

CHAPTER 10

***Phalaris arundinacea* Control and Riparian Restoration within Agricultural Watercourses**

10.1 Introduction to RCG Control and Restoration (Goal 9)

The agricultural industry in western Washington can be traced back as early as the 1820s (Kantor 1998). A majority of the agricultural land within King County lies within river valleys and floodplains which are subject to frequent flooding and saturated soils. The watercourses utilized for drainage of the floodplains typically flood due to the accumulation of fine sediment, associated with the spread of the invasive grass species, *Phalaris arundinacea* (reed canarygrass (RCG)). This in turn, leads landowners to clear the channels by dredging, a practice which has important implications for salmonid rearing habitat.

The re-establishment of vegetated riparian buffers along agricultural watercourses is a significant challenge, in large part due to competition by dense monocultures of RCG. In addition to constricting watercourses, RCG does not provide sufficient shade or instream habitat structure in the form of large woody debris (LWD) needed to constitute high quality riparian and in-stream habitat. Furthermore, it is believed that RCG may harbor a different and perhaps less desirable assemblage of invertebrates when compared to native woody streamside vegetation. Thus, finding effective and economical control measures for reed canarygrass is imperative for these watercourses.

Reed canarygrass can alter the surrounding habitat by: 1) constricting flow in watercourses; 2) filling shallow lakes and ponds, degrading fish and wildlife habitat; 3) greatly increasing evapotranspiration, which can affect local shallow groundwater characteristics (Antieau 1998); and 4) arresting natural plant succession on the site (Antieau 2002). These alterations result in a complete passage blockage for anadromous salmonids during a portion of the year, generally late-summer and early-autumn (Carrasco 2000, and personal observation).

As discussed in Chapter 1, the overall objective of this entire study was to determine effective and economical means to maintain agricultural watercourses while protecting fish habitat as described in the Sampling and Analysis Plan developed by Washington State University and the University of Washington (2006) and approved by KCDNRP. In support of that mission, the goal of this chapter was to determine successful methods for RCG control and eradication.

10.2 Study Background

10.2.1 Reed Canarygrass Biology and History

Phalaris arundinacea is a C3 cool season grass, a perennial with robust, hollow culms that reach up to 2 meters tall. These stems average one cm. (.4 inches) in diameter, with a reddish tinge at the top during the growing season. The leaf blades are flat with prominent ligules. This species spreads predominantly by creeping rhizomes which can be stout with 6-10 nodes (Comes 1971). RCG is an obligate outcrosser due to self sterility (Lavergne and Molofsky 2004).

Reed canarygrass is one of 15-20 species of this genus distributed throughout the world within the northern temperate regions of 5 continents. It is reported to tolerate annual precipitation of 3-26 cm. (1.18-10.2 inches), annual temps of 5-23 C (41-73.4 F), and a soil pH of 4.5 to 8.2. RCG does not however, perform well in subtropical or tropical climates. Southern Virginia marks it southern boundary on east coast and across to southern California on the west coast (Lyons 2002).

Many researchers consider RCG to be native to the inland Pacific Northwest (PNW), Europe and Asia, while others reason that it was introduced from Europe. A third view is that the aggressive North American genotypes are hybrids of native populations and the introduced European cultivars (Merigliano and Lesica 1998).

Early collectors found RCG throughout the inland northwest between 1825 and 1911. Ten specimens predate Euro-American settlement in that region or were collected from remote, undeveloped areas. Of the six specimens from riverine habitats, five indicated that RCG was abundant while the three from meadows and springs indicated that the plant was uncommon or rare (Merigliano and Lesica 1998).

RCG is known to have three cytotypes in Eurasia, mostly represented by an allotetraploid, with the subspecies name of *arundinacea*, along with a hexaploid form, subspecies, *oehlerii*. There is also a diploid cytotype, *rotgesii*. Merigliano and Lesica (1998) stated that the herbarium specimens most closely resembled the diploid, but recent evidence by Lavergne and Molofsky (2004) shows that the invasive occurring in Vermont and NC, are tetraploid and more similar to the cytotype in Eurasia.

There have been documented repeated introductions in the US of numerous RCG cultivars for a variety of purposes. Cultivation for agronomic purposes began in Sweden in 1749. The first trials in the United States took place in the mid 1830's, using the *picta* form because of the higher palatability. During the 1850's, RCG received a great deal of attention for reclamation projects and was recommended for reclaiming peatlands and marshes. Most of the stands on the Pacific coast are attributed to a cultivated stand in Coos County, Oregon established in 1885 (Merigliano and Lesica 1998; Comes 1971).

This species is listed as a noxious weed by the US Federal government and is a class C noxious weed in Washington State. It is a notorious global weed as well, cited as a serious or principle weed in numerous countries throughout the world.

Ecologically, RCG has the ability to exclude native species through competition. This species is extraordinarily successful at out-competing other vegetation due to several factors. There are no known dormancy requirements, and the seeds germinate immediately after ripening with a very high (97%) viability rate (Apfelbaum and Sams 1987). However, the primary means of reproduction is by vegetative growth, i.e., spreading by aggressive rhizomes and stems (Naglich 1994). Each plant can produce a dense mat of rhizomes within one growing season (Apfelbaum and Sams 1987), and even seven to eight week old seedlings produce these rhizomes (Crockett 1996). RCG can reach heights of six feet or greater (Antieau 1998), easily shading out smaller, slower-growing shrubs and tree saplings.

Reed canarygrass is also well known for slowing water velocities, thereby inducing sediment deposition and resulting in a positive feedback loop of more flooding and increased sedimentation rates within affected channels. This is in large part due to the density of the shoots and is increased by a dense shallow root mass. A study of RCG growth characteristics found that at least 88 percent of the emergent shoots on established plants in the field originated from rhizome or tiller buds located in the top 5 cm (1.9 inches) of the soil (Apfelbaum and Sams 1987).

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As noted above, the aggressive RCG found in the Midwest and PNW may be a hybrid or hybrids of the native and introduced cultivars. Generally, hybridization events allow for the rapid reshuffling of varying adaptations. Elements of an entirely foreign genetic adaptive system can be carried over into a previously stabilized one. Each hybrid produced by these species may produce different recombinations, each of which may be able to adjust to different niches. The ever increasing heterozygosity brought in by hybridization would be capable of producing increased variation generation after generation. This genotypic diversity would confer an advantage as the different genotypes could vary in response to environmental influences and new niches which would allow a selective advantage for the hybrids (Anderson and Stebbins, Jr., 1954). Hence, a hybridization event with RCG would allow a mixture of a native that has become very well adapted to the environmental conditions within the PNW with cultivars that have been bred to be vegetatively vigorous and drought tolerant.

Ellstrand and Schierenbeck (2006), presented evidence with 28 examples of hybridization events which preceded invasiveness, such as with *Spartina anglica* and *S. alterniflora* with producing *S. foliosa* in CA, *Typha x glauca*, and some of the *Tamarix* spp.

The success of reed canary grass within various environments may be due to both genetic diversity and phenotypic plasticity. In a study by Coops et al. (1996), biomass allocation patterns of RCG changed in response to vegetative cover. RCG allocated more resources to belowground biomass when grown within dense vegetative cover. This is probably giving the plant a competitive advantage in the next growing season, as it over-winters as root stock and is one of the first perennials to emerge in the spring (usually late winter here in the PNW). This morphological plasticity was also important for surviving within various water depths. Plants grown in deeper water allocated more biomass to elongating the stem, while plants grown in up to 5 cm. (1.9 inches) of water allocated more biomass to the roots (Coops et al. 1996).

Maurer and Zedler, 2002 also found morphologically plastic behavior by RCG when testing root: shoot ratios and the lateral expansion rates in different nutrient conditions. RCG spread nearly 50% farther and produced twice as many tillers under high nutrient conditions and produced fewer tillers closer to the parent clone under low nutrient conditions. This combination of the “guerilla” and “phalanx” strategies (consolidation strategy) allows RCG to dominate the vegetation year after year by maintaining their position in poor conditions and/or years and spreading into new areas during high nutrient conditions and favorable years. High levels of genetic diversity increase the likelihood that a particular genotype will flourish and spread into new areas. Thus, genetic diversity coupled with suitable environmental conditions frequently enable reed canary grass to aggressively take over entire plant communities.

The plant architecture of this species may also play a significant role in its competitive abilities. Grime and Hodgson (1987) listed characteristics of species with high competitive ability: “(1) a robust perennial life form with a strong capacity to ramify vegetatively, (2) the rapid commitment of captured resources to the construction of new leaves and roots, (3) high morphological plasticity during the differentiation of leaves and roots, and (4) short life spans of individual leaves and roots”. Gaudet and Keddy (1988) found that tall shoots, leaf shape (length:width ratio), and large canopy diameter were morphological characteristics that were significantly correlated with increased competitive ability in wetland plants.

The horizontally oriented leaves and tall culms of RCG improve the efficiency of light utilization. A study by Wetzel and van der Valk (1998) found that *Carex stricta* and *Typha latifolia* were both heavily impacted when grown with RCG. In this study, RCG maximized the capture of light and nutrient resources by maximizing vegetative growth, even under low nutrient or soil moisture conditions. RCG is a superior competitor, regardless of the environmental conditions as predicted by Grime (1979).

Additionally, Pysek (1997) indicated that invaders that reproduce predominantly by vegetative means, clonal species, generally have a greater ecological impact on native communities than non-clonal species. Once established, clonal species can persist and spread into conditions that are more stressful than those where it colonized (D’Antonio 1992).

In spite of decades of study, there is currently no comprehensive strategy for the effective removal of existing RCG and establishment of alternative native vegetation (Perry and Galatowitsch 2004; Perry et al. 2004; and Forman et al. 2000). The management techniques utilized to date include chemical control (glyphosate), mowing and grazing, excavation of the

substrate, water level manipulation, micronutrient management (boron), macronutrient management (nitrogen), burning, and shading (black plastic mulching and/or competitive exclusion).

Reed canarygrass responds quickly after mechanical removal by growing back from rhizomes and seeds remaining in the soil (Apfelbaum and Sams 1987). However, repeated shoot removal damages plants via stress when disturbance events are frequent. Available carbohydrate reserves are greatest during the winter months, declining to a low point in mid-summer. Depletion events happen as the growing point is elevated in spring and as the seed heads develop in early summer.

10.2.2 Objectives

The objectives of the riparian vegetation enhancement section of this project include:

- a) finding a BMP protocol for the effective control/eradication of reed canary grass, and
- b) determining a method for providing native ground cover and woody riparian vegetation that is vigorous, shade producing and provides habitat for insects that constitute prey for salmonids.

The first objective was addressed within a Pilot Project which was implemented in the fall of 2002 and spring of 2003. Response data was collected throughout the spring, summer and early fall of 2003. This objective was resolved by answering the following questions:

1. Will the application of steam provide a significant kill of the RCG?
2. Do the allelopathic plants, *Gaultheria shallon* and *Trifolium repens/pratense* effectively compete with reed canary grass?
3. Will an allelopathic mulch placed on top of RCG successfully suppress RCG?
4. Will shading RCG with a heavy opaque material effectively suppress the RCG?

The second objective, the planting of a native woody species for shade of the watercourse, has been integrated into the treatments that were deemed “successful”; i.e., those with significant RCG eradication. These new treatments have been applied within the Principal Project which was implemented in the fall of 2003.

From observations based on the pilot study results, the following research questions will be addressed:

1. Will the following treatments negatively affect the density of reed canarygrass?
 - a) burlap/compost layers densely planted with native species providing multiple canopies (RCG barrier)
 - b) red cedar hogfuel densely planted with willow species (*Salix sitchensis*)
 - c) red cedar hogfuel placed on top of RCG with the RCG barrier on top of the hogfuel

2. Is *Scirpus microcarpus* an effective competitor with reed canarygrass?
3. How long will reed canarygrass rhizomes need to be covered with a weed block fabric to deplete the carbohydrate reserves?

10.3 Pilot Project

10.3.1 Pilot Project Rationale

Steam has been proven to be an effective treatment for numerous weedy species (Quarles 2001). In most cases, the efficiency of steam has been equal to the use of herbicides. Most annual species are killed immediately, however, as with herbicide, perennial species typically require additional applications. The use of steam has not been attempted on RCG to date. Most studies have been completed with a system from the Waipuna Company, whose steamers reach a temperature of 98° C (~208° F). This study employed a steamer that was programmed to reach much higher temperatures. The steam was at 149° C (300° F) within the pressurized machine and exit the hose at 138° C (~280° F).

The native shrub, *Gaultheria shallon* (salal), is an allelopathic plant that releases an allelochemical called tannins from the flowers, leaves and roots (Preston 2002). Various studies have indicated that salal has a negative impact on the re-growth of conifer seedlings after logging (Preston 2002), this is being called a “salal complex” (Province of BC). Tannins are able to bind proteins in a manner that negatively impacts the availability of nitrogen (Cornell University 2001). Salal is being utilized in this study in order to gauge the impact that tannins and nitrogen reduction may have on the re-growth of the RCG. Salal is also an evergreen and could provide year round shade within the drier areas of the agricultural waterbodies, along the top of the bank where the soil is generally much drier, especially during the later part of the growing season. Additionally, a variety of wildlife species consume the leaves, flowers and berries of salal (Province of BC).

Clover is being tested due to a direct observation from a site visit during the summer of 2002 where it seemed to be surviving in the presence of surrounding RCG. Planting fast growing cover crops to compete with and suppress aggressive invasive species while the desired species become established has been utilized in some agriculture and prairie restorations (Perry and Galatowitsch 2003). Gunti et al. (1999) found that red clover (*Trifolium pretense*) reduced the biomass of the invasive hedge bindweed (*Calystegia sepium*) within a greenhouse experiment.

Adding carbon sources have been proven to have a negative effect on the nitrogen availability within the soil where they have been applied in addition to the impact of shading the weed (Stout 2002; Duryea et al. 1999). Mulch has been utilized anecdotally, however, the use of a mulch, especially one with allelopathic tendencies, has not been reported from a scientific study. The use of a red cedar hogfuel mulch applied ~25 cm (9.84 inches) will be tested in this experiment.

Shade material has been frequently utilized to control weeds. The leading drawback with using most shading fabrics with a species such as RCG is the propensity for the material to either break

down as a result of prolonged exposure to sunlight, allowing the re-growth of RCG from underground rhizomes and seed, or that the material is too light to remain in place, thus allowing RCG re-growth from underneath. RCG stores an extensive amount of carbohydrates within the rhizomes. Therefore, simply using a typical manufactured shade cloth has not been shown to be effective over the long term. Utilizing old carpet was tested in this study due to the weight of the material and resistance to break down in ultraviolet light. However, new types of geotextile fabric are being considered for testing the effects of shade-producing materials at an additional set of study sites.

10.3.2 Pilot Project Methodology

The goal of this research project was to develop RCG suppression methodologies that would be valid throughout the entire study area (Figure 10-1). As a part of this research project, a successful method for reducing the vigor of RCG and eventually removing RCG was investigated in an initial pilot study in which separate test plots were established to evaluate the impact of various cover and treatment alternatives for one season. The Pilot Project took place along a watercourse at the Quigley property within the Sammamish APD–Woodinville, Washington (Quigley A, Figure 10-2). Each plot was placed linearly along a ditch at the pilot study project site. Each plot is one treatment cell (subplot) wide and 10 cells long. There are three replicates of these 10 treatment cells. The cells are 1.5 meters by 1.5 meters. The RCG within all three plots and all treatment cells was mowed and tilled to remove the aboveground biomass and loosen the rhizomes in the top ~15 cm (5.9 inches) of soil. A trench of ~30 cm (11.8 inches) was placed around each cell and a rhizome barrier placed in each trench to remove any rhizomatous connection with the surrounding parental clones.

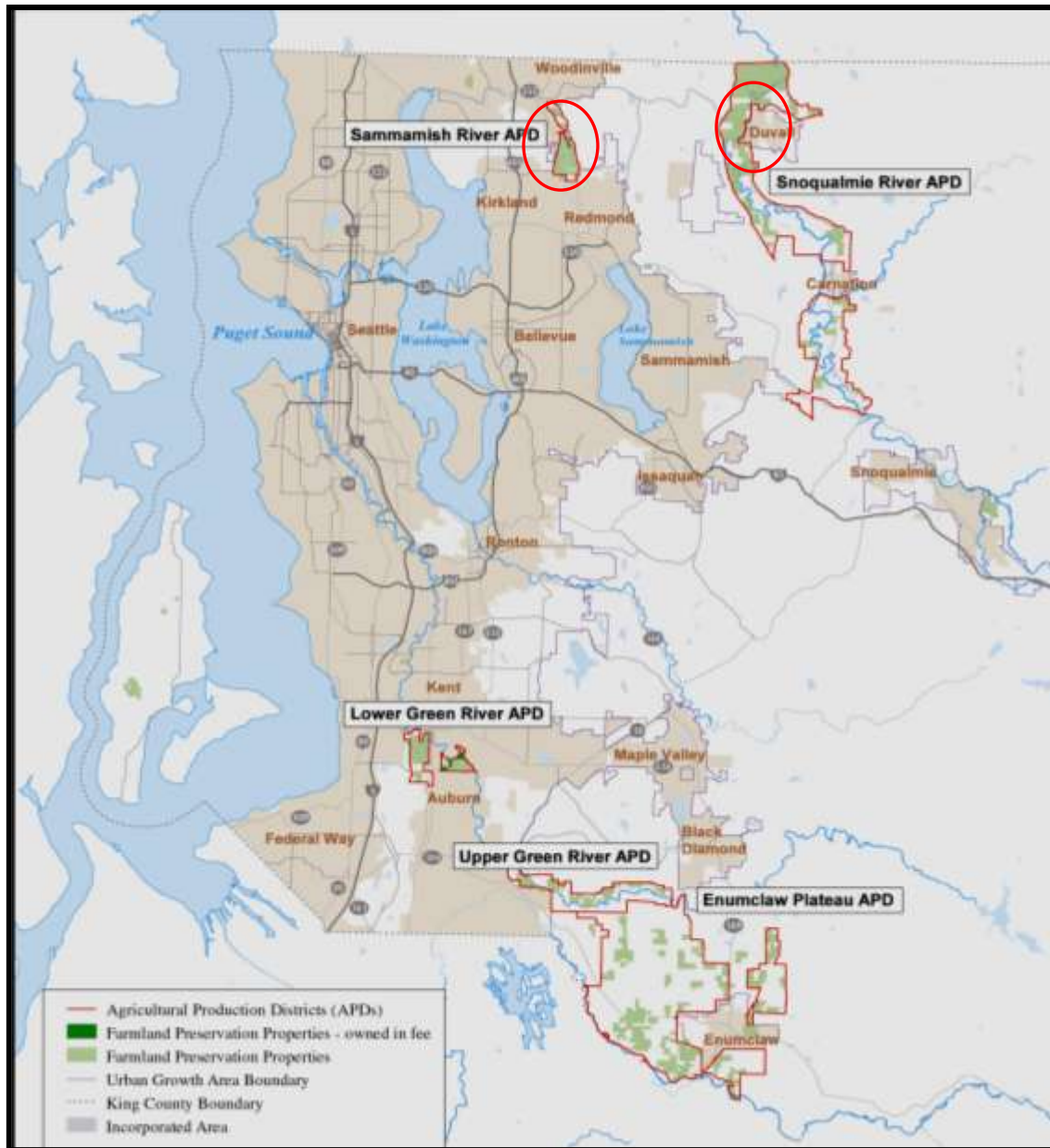


Figure 10-1. Overview of study area.

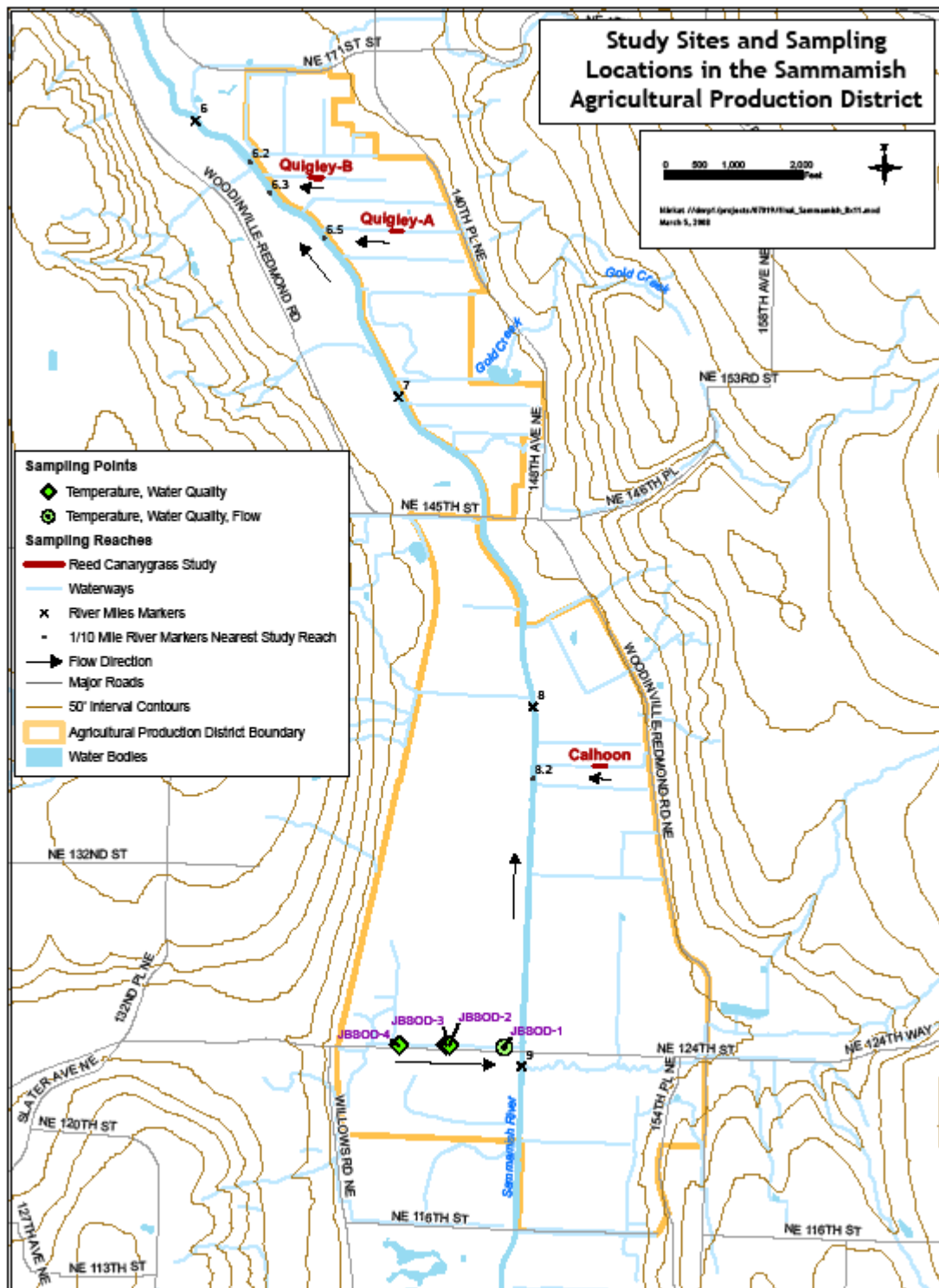


Figure 10-2. Aerial photograph of Woodinville horticultural (Quigley) and livestock (Calhoon) farm study location.

The treatments were assigned randomly within each replicated plot. Examples of the hypothesized treatments are listed below in Table 10-1, and the layout for the project at the site can be found in Appendix 10-A.

Table 10-1. Treatment design

Steam

Red Cedar Hogfuel	Densely Planted Salal	Cover Crop	Shade	Control
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No Steam

Red Cedar Hogfuel	Densely Planted Salal	Cover Crop	Shade	Control
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The Steam Machine (Stinger 1) was mounted on the back of a pickup truck, along with a generator and a water tank. The water was heated within the steamer and steam was sprayed on the plots assigned to this treatment for a designated period of time. After one week, the additional treatments noted in Table 10-1 were installed.

The cells assigned to the densely planted salal treatment were divided into 10 cm x 10 cm (3.9 inch) squares. One salal plug was planted within each 100 cm² area. Those cells designated for the cover crop treatment had *Trifolium repens/pratense* seeds spread on top at a rate of ~226.8 grams per cell. A summary of the planting schedule is shown in Table 10-2.

Table 10-2. Planting schedule for the treatment plots

Common Name	Biological Name	Size/Type	Plants per cell
Salal	<i>Gaultheria shallon</i>	Plugs	90 plants 1 per 10x10 cm square
Clover, red and white	<i>Trifolium repens/pratense</i> mix	Seed	0.5 lbs per cell

The allelopathic mulch composed of red cedar hogfuel was applied in an undecomposed condition in order to maximize the concentrations of allelochemicals within the wood and bark. A 28 cm (11 inch) layer of mulch was placed on top of the cells.

The shading material utilized for this pilot project was nylon carpet cut into 2.25 meter squares to test whether carpet material would provide both the strength and weight needed to suppress the re-growth of RCG rhizomes. This fabric was used due to the low cost (free).

Additionally, three nitrogen reduction treatments were applied at the pilot study site in March, 2003. Initially, 1.5 pounds of sucrose was applied to the cell after the above ground new growth was removed by shears. Ten cm (3.9 inches) of sawdust was incorporated as a carbon source. In

accordance with a nitrogen reduction study protocol used in the Willamette Valley (Davis 2000), the treatment was then reapplied later in the growing season, in early May. This reapplication included five pounds of sucrose placed into 25 holes within the initial layer of sawdust using a large PVC pipe. An additional 15 cm (5.9 inches) of sawdust was added on top, totaling 25 cm (9.8 inches) of sawdust.

RCG stem densities were measured bi-weekly to determine the success of each applied treatment. A one meter square was placed in the middle of each treatment (dowels were permanently placed in each cell to verify that the measurements were taken in the same place), and the stem density of the RCG was determined throughout the spring and summer of 2003.

10.3.3 Conclusions from Pilot Project

Soil core samples were taken from the soil under the mulch and nitrogen reduction treatments along with the control plots at the end of the summer, 2003. These samples were dried and sent to the University of Massachusetts soil laboratory for testing. The results indicated that the nitrogen was almost the same for the soil under the treatments versus the control plots.

Stem count data from the growing season of 2003 indicate that two treatments were particularly successful (Figure 10-3). The hogfuel and shade material treatments (whether used with or without steam) suppressed the reed canarygrass significantly when compared to the control plots and the other treatments. These two treatments have been expanded upon and utilized within the Principal Project discussed below.

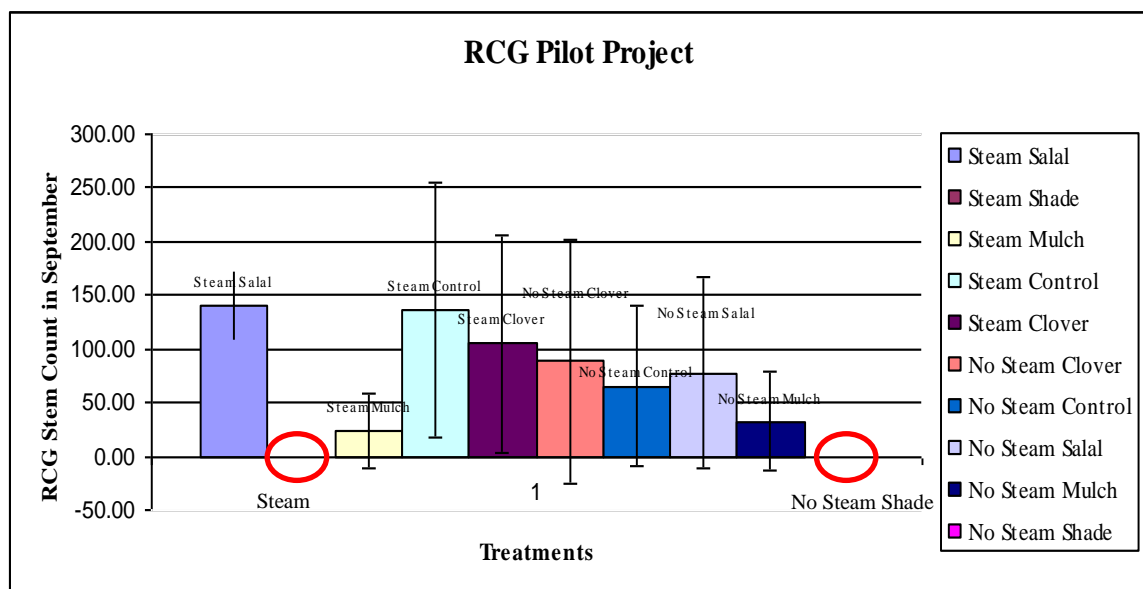


Figure 10-3. Pilot project results from 2003

10.4 Principal Project

10.4.1 Principal Project Rationale

Green and Galatowitsch (2002) found that agricultural runoff and the associated nitrogen addition contributes to the increasing colonization and dominance of reed canary grass. After testing three comparable levels of N on RCG and native species, they found that the total shoot and root biomass of the native community was suppressed with RCG, at all levels, and that shoot growth of the native community was reduced by nearly one-half at the highest N level. Kercher and Zedler (2004) had similar results with inorganic nitrogen additions. RCG reduced a native sedge biomass by 91% while the sedge did not impact the RCG. In contrast, in a carbon enriched soil, the competition by the sedge reduced the RCG biomass by 82% while RCG competition reduced the sedge biomass by only 32%.

Adding carbon sources has been proven to have a negative effect on the nitrogen availability within the soil where they have been applied in addition to the impact of shading the weed (Stout 2002; Duryea et al. 1999). There have been many successful studies utilizing some form of carbon, generally, wood chips and/or sawdust and sucrose to reduce nitrogen trying to give native species an competitive edge on exotics (Reever and Seastedt 1999; Blumenthal et al 2003). However, these have generally been practiced in prairie and grassland systems (Corbin and D'Antonio 2004). Davis 2000, found that carbon additions were effective in suppressing weed biomass and promoting native species within a wetland prairie system in Oregon. Generally, the optimum carbon to nitrogen ratios is around 10:1. Examples of a few amendments include: corn stalks = 60:1, sawdust (weathered 2 months) 625:1 and Douglas fir bark 491:1. The use of sawdust and sucrose and red cedar hogfuel was attempted in the pilot study. The use of red cedar hog fuel was tested in conjunction with other methods of treatment in the principal project.

Shade material has been frequently utilized to control weeds. There are several inadequacies when using shading fabrics with a species such as RCG. Most fabrics have the tendency to break down as a result of prolonged exposure to sunlight, allowing the re-growth of RCG from underground rhizomes and seed. Additionally, typically the material is too light to remain in place, thus allowing RCG re-growth to literally push up the fabric from underneath due to the extensive amount of carbohydrate storage within the rhizomes. Therefore, simply using a typical manufactured shade cloth has not been shown to be effective over the long term (personal observation 2005). Placing a layer of burlap (an inexpensive, often free fabric) on top of mowed RCG, with compost on top (for planting in) and another layer of burlap on top of the compost, should provide sufficient weight to suppress the RCG underneath.

Mixed canopy layers allow for a reduced transmittance of light with a lower red:far-red light ratio than direct sunlight. Lindig-Cisneros and Zedler (2001) exposed RCG seeds to low red:far-red ratios and found that germination decreased by nearly 30 percent. Canopy gaps were shown to increase invisibility in this study, as reed canarygrass did not germinate in no-gap treatments, regardless of species richness. Reed canarygrass did germinate under a canopy with only 1-species, but was 43 percent lower in mixed canopy treatments.

However, in a greenhouse experiment with three-month-old reed canarygrass clones, Maurer and Zedler (2002) tested the effects of shading (47, 67, and 86 percent treatments) on the expansion of new tillers. When tillers were attached to un-shaded parental clones, new growth was not significantly affected. After an invader establishes, the shade cast by neighboring plants may no longer inhibit growth or vegetative spread.

10.4.2 Principal Project Methodology

The Principal Project research was conducted at three field site locations. The three locations chosen for the study were: 1) an agricultural watercourse on the Olney property in Duvall, WA, 2) a horticultural watercourse on the Calhoon property at 13651 Redmond-Woodinville, WA, and 3) an additional agricultural watercourse at the Quigley property. The general locations of the study sites were shown previously in Figure 10-1. These sites were chosen due to the occurrence of dense swards of RCG along the agricultural water body, ease of access, and the lack of planned dredging by the other researchers participating in this project. The study sites also represented three different farm types and two different Agricultural Production Districts (APDs) within King County. These are an agricultural farm (“Quigley” property, Woodinville, Sammamish APD) (Figure 10-2), a livestock site (“Calhoon” property, Woodinville/Redmond, Sammamish APD) (Figure 10-2) and a “natural” site (Duvall, Snoqualmie APD; Figure 10-4).

Based on the observations cited in the rationale section, the plant species used for the RCG barrier treatment were chosen due to their ability to: a) provide two or three canopy layers at any point within the plot, ie., their height; b) emerge early in the season, the emergent, *Scirpus microcarpus* placed closest to the watercourse will emerge early in the season as does RCG; and c) tolerate wet conditions when placed close to the watercourse, gradually changing to dryer conditions when placed top of the bank.

Wipfli (1997) found that terrestrial invertebrates were as important for certain salmonid species as aquatic invertebrates during the spring, summer and fall and that the riparian vegetation influences the terrestrial invertebrates available for salmonids. A compact and diverse lower canopy layer with a broadleaf alder upper canopy provided a more diverse and productive terrestrial invertebrate community. Alan et al. (2003) went further in looking at specific over and understory species and their contribution of invertebrate mass. Salmonberry (*Rubus spectabilis*), currant (*Ribes sp.*), blueberry (*Vaccinium ovalifolium* and *V. alaskaense*) and alder (*Alnus rubra*) were all noted as contributing a high level of invertebrate biomass, many of the taxon being those that were also found in the stomach samples of juvenile salmon. Alder was not used in this project as it is a nitrogen fixer and RCG is understood to be a nitrophilic plant, yet alder could be used after the RCG is under control or in other re-vegetation situations.

These three species (*Rubus spectabilis*, *Vaccinium ovalifolium* and *Ribes bracteosum*) were therefore chosen due to the invertebrates that they have been shown to provide and will be utilized within the “RCG barrier” treatment of the principal project are shown below in Figure 10-5.

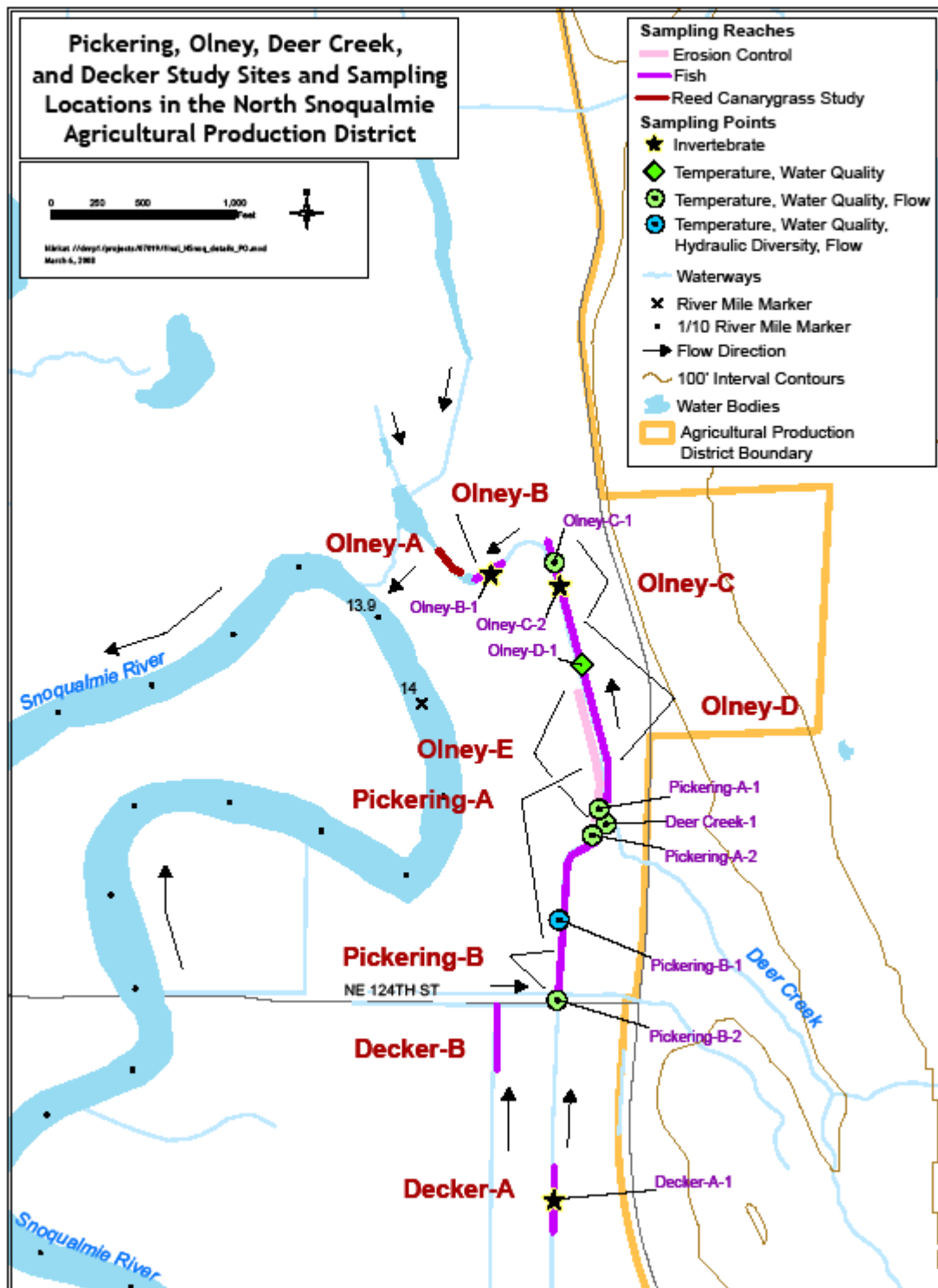


Figure 10-4. Aerial photograph of Duvall study location.

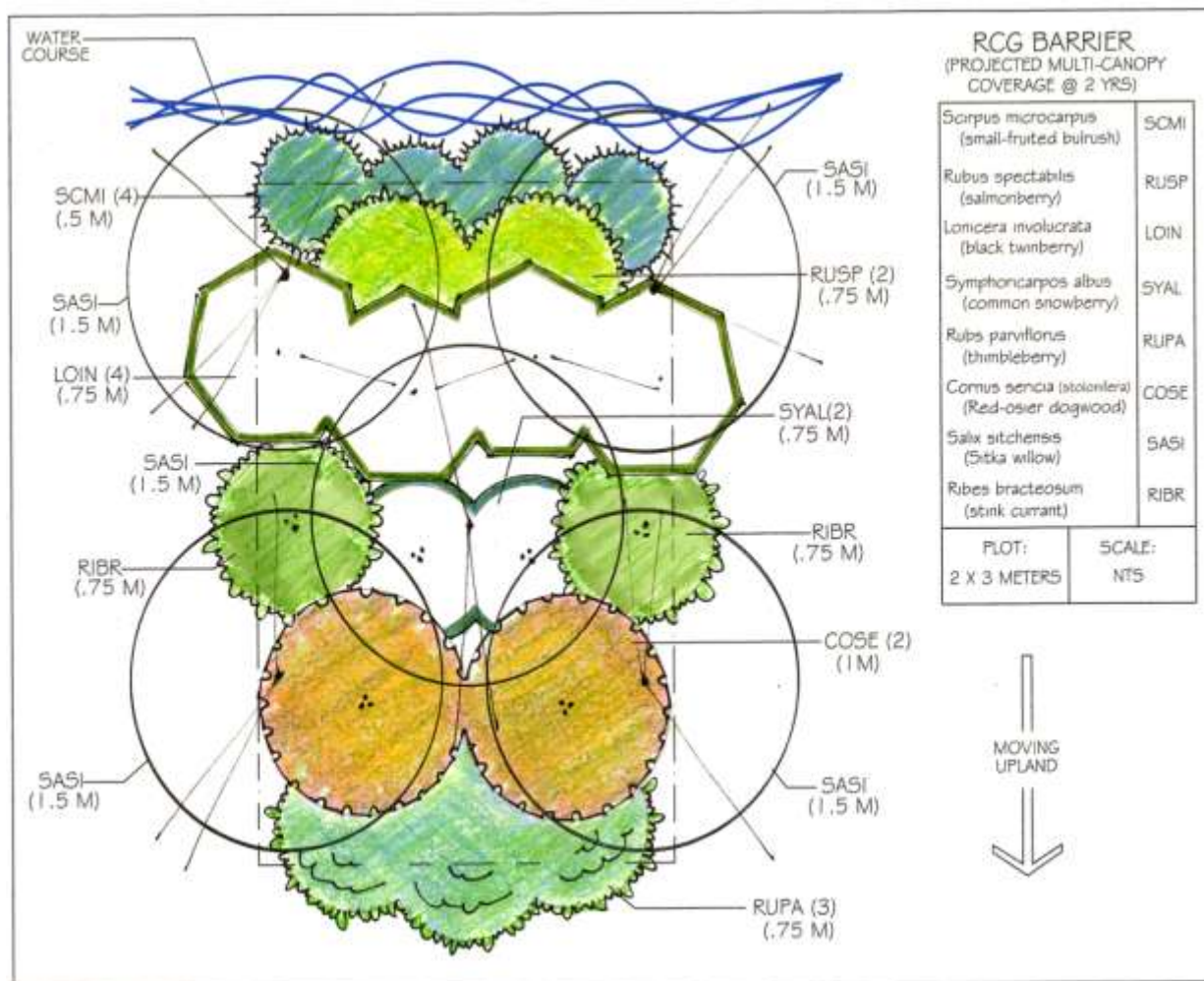


Figure 10-5. Spacing and design for each “mat” treatment plot

Treatments for the Principal Project involved utilizing a RCG barrier that was developed specifically for this study. Burlap fabric was placed on top of mowed RCG and compost (15 cm deep, 5.9 inches) was placed on top of the fabric within two by three meter plots. Burlap fabric was then placed on top of the compost and staked on each side. The burlap/compost “pillows” were used due to the weight and shade that they provided (as shown by the carpet in the pilot project to suppress RCG) as well as the ability to plant within the compost. Additionally, when plants or stakes are placed within the fabric, RCG competes with the plant/stake, and grows up through the hole and again on top of the fabric. With this design, the RCG underneath the mat will not be exposed for several years and is not capable of competing with the more desirable planted vegetation. Therefore, RCG seed and rhizome fragments from elsewhere are the chief concern for re-growth on top of the mat. In accordance with the second objective, b, and in order to provide a habitat which will be competitive with the RCG seeds and rhizome fragments, plants

were chosen for re-vegetation that provided the maximum shade possible and numerous canopy layers.

In addition to the RCG barrier treatment, the two additional treatments tested within the Principal Project are:

- 1) 25 cm. (9.8 inches) of red cedar hogfuel with 3 foot willow stakes placed within at a density of 12 stakes per plot (1.5 foot on center), and
- 2) 10-15 cm. (3.9-5.9 inches) of red cedar hogfuel with the RCG barrier placed on top.

Willows were chosen due to their proven ability to survive any fungal growth from being placed within the mulch and the shade produced (Chalker-Scott personal communication 2002). The three treatments (plus one control) for the principal project are illustrated below in Figure 10-6. There were three replicates of the four plots at each of the three study locations.

As with the other treatments in this study, the original biomass of the RCG was mowed before the placement of the treatments. Plots at the Olney (natural site) and Quigley (agriculture site) properties were 2 x 3 meters and the plots at the Calhoun (livestock site) property were 1 x 2 meters. As with the pilot project, stem density was measured bi-weekly beginning in the spring of 2004 and continuing through the end of 2005, to determine the success of each applied treatment within the principal project over two growing seasons. Appendix 10-A displays the treatment arrangement at each site.

Treatments – A randomized block experimental design was implemented with 3 treatments (plus control) within 3 blocks (or replicates) chosen randomly at each site, or 9 replicates of each treatment in total. Reed canarygrass was mowed with string trimmers and a rhizome barrier was placed around 1.5x2.5 meter plots at the agriculture and natural site and 1x1.5 meter plots at livestock site. The treatment plots were placed linearly along the watercourse at each site.

Treatment #1 - Burlap fabric was placed on the mowed RCG with 25 cm (9.8 inches) of compost and another layer of burlap placed on top with a designated planting design (see Figure 10-6) for the RCG barrier treatments.

Treatment #2 - Burlap fabric was placed on top of the mowed reed canarygrass with 25 cm (9.8 inches) of hogfuel and another layer of burlap on top with 3 foot willow stakes placed within at a density of 12 stakes per plot for the hogfuel willow treatment.

Treatment #3 - 25 cm (9.8 inches) of hogfuel will be placed on top of the reed canarygrass with the same RCG barrier treatment described above placed on top of the hogfuel for the hogfuel/RCG barrier treatment.



Figure 10-6. Examples of treatments used in study

Data Collection - The stem count, or reed canarygrass re-growth, for each treatment and the control plots was counted bi-weekly throughout the growing season (February through November) for two years. Percent cover of vegetation within each plot was recorded at the end of the second growing season to determine survival and competitive efficacy of the planted native species with the RCG.

10.5 Competition Experiment Objectives

10.5.1 Rationale for Competition Experiment

Field observations from both the experimental field sites and in some natural areas within King County indicate that *Scirpus microcarpus* (small fruited bulrush, SFB) is thriving and competing effectively with *Phalaris arundinacea* (RCG). Due to these field observations, a competition study between *Phalaris arundinacea* and *Scirpus microcarpus* to test the competitive abilities of SFB with RCG in a controlled setting was performed in the greenhouse at the University of Washington's Center for Urban Horticulture.

The objective of the greenhouse competition experiment was to determine if SFB is an effective competitor with the aggressive, invasive RCG. An "effective competitor" was defined as a species that negatively impacted the performance of its neighbor by limiting growth. In this case, limiting growth is considered the reduction of above ground and/or belowground biomass of RCG when grown with SFB (with inter-specific competition) versus with intra-specific competition. In this experiment, resource competition was examined.

The ecosystem in which the field experiment for this research is taking place is a high resource ecosystem (agricultural fields with drainage watercourses). This system experiences generally high levels of nitrogen from fertilizer and livestock and high soil moisture levels close to the watercourse and high levels of disturbance due to mowing and flooding. It is assumed that the species with high relative growth rates will be the most effective competitors as rapid growth would allow them to dominate the available space, produce more biomass and acquire the most resources while doing so.

While looking at competition intensity and asymmetry, Johansson and Keddy (1991) found that the intensity of competition increases with species that are morphologically more similar and similar individuals have more symmetrical interactions. Biomass, especially above ground biomass, was determined to be the plant trait that most strongly correlated with competitive ability and the suppression of the phytometer used in their study (*Lythrum salicaria*). Below ground biomass, plant height and canopy area (cm²) were also highly correlated (Gaudet and Keddy 1988).

RCG and SFB are both long lived perennials which are highly rhizomatous, similar in height (up to 2 m for RCG, up to 1.5 m for SFB), have similar rooting depth (25 cm (9.8 inches) for RCG, 30 cm (11.8 inches) for SFB) and similar specific leaf area ratios.

The goal of this restoration project was to control RCG along and within watercourses on agricultural land in King County. Within these systems, the RCG is mature, heavily clonal and in most every case, a monoculture on the site. Hence, testing the competitive abilities of the seedlings of these two species does not coincide with the current conditions of the sites nor the conditions found for the majority of the restoration projects in the PNW. Therefore, bare-root plants for each species were utilized for this experiment.

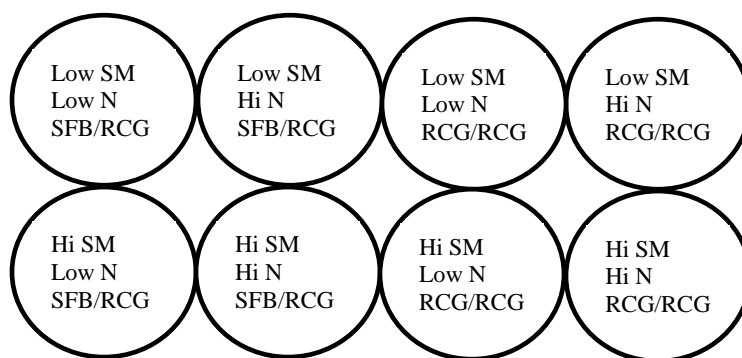
10.5.2 Experimental Design of Competition Study

Initially, 60 SFB plants were purchased and 140 RCG plants were collected from the field. The plants were chosen based on similarity, via number of nodes and similar root length. Ten plants were randomly chosen for an initial dry weight analysis. This data was compared with the mean dry weight analysis for each treatment at the end of the study. Of the remaining plants, 4 SFB and 12 RCG were randomly chosen for each treatment, the above ground stems were clipped at 10cm (3.9 inches) and the plant was weighed. Past experiments have shown that the above ground biomass (stem length) of the plants received can be quite variable and generally die immediately after planting and therefore, were removed before weighing and planting. After weighing, the clipped plants were assigned a number for tracking purposes. This information was used to determine if there was a correlation between the initial fresh weight of each plant and the final biomass.

An additional tagging method was utilized within the control pots (RCG/RCG) where one of the RCG plants was randomly chosen to be the RCG plant weighed at the end of the experiment. Using bare-root plants that were of a similar size based on number of nodes, root length, and same length of above ground biomass reduced the variation at the beginning of the experiment.

Randomly choosing which plants were to be placed in each treatment provided a Gaussian distribution among the treatments.

Each two gallon pot with either two RCG plants or one RCG plant and one SFB plant was placed independently within its own tray for watering and fertilizing. The trays were placed in a complete randomized block design on a table within zone 2 of the CUH greenhouse (~21°C daytime, ~17°C night, 14 hour photoperiod/1/8 full sunlight). There were 10 blocks with one of each of the eight treatments placed randomly in each block. Figure 10-7 illustrates the typical layout of each block.



SM = soil moisture; N = nitrogen; SFB = small fruited bulrush; RCG = reed canarygrass

Figure 10-7. An example of the eight treatments within one of the ten blocks

10.5.3 Treatments

Nutrient, Soil Moisture and Competition - Along with the observations that the planted SFB was thriving and competing with RCG, it was also observed that SFB was most successful when placed closer to the watercourse and within what was presumed to be a site with higher nitrogen concentrations due to adjacent livestock farms. Additionally, the competitive ability of a species typically changes with differing nutrient and environmental conditions, the neighboring species and developmental stage. In addition to testing the competitive abilities of SFB with RCG, both species were placed within two soil moisture and nitrogen regimes. Table 10-3 summarizes the experimental analysis. This allowed an opportunity to determine if SFB was as competitive with RCG within a wide range of environmental and nutrient conditions or only within particular conditions and therefore, more site specific.

Based on the mean of three soil samples from the livestock site in Woodinville with the NH_4 and NO_3 added together, the high nitrogen scenario for this experiment represented 66.08 mg (.002 oz) per kg. The low nitrogen level was defined as 9.46 mg (.00033 oz) per kg based on soil samples from the “natural” site in Duvall.

Table 10-3. Competition under various nutrient and soil moisture regimes

FACTORS →	Nitrogen	Soil Moisture	Competition
Level 1	High	High	Intraspecific (RCG/RCG)
Level 2	Low	Low	Interspecific (RCG/SFB)

For the high soil moisture regime, the water level within the tray was kept at a depth of approximately 2.5 cm (0.98 in.). The low soil moisture regime was determined by averaging the precipitation data from the Monroe and Tolt South Fork Reserve weather stations (Monroe for it's proximity to the Sammamish sites and Tolt for it's proximity to the Snoqualmie site). Data for typical summer months (June through August) averaged over the last six years (2000 through 2005) was used. The average rain per day for the two sites was 2.15 mm (0.0846 in.). Adjusting this to the surface area of the pots utilized for this experiment allowed for 800 mL (1.69 pints) of water per week for the low soil moisture treatment watering regime.

The plants were planted immediately, two RCG plants per pot (size two pots) for the control pots and one RCG plus one SFB plant placed together for the competition treatment (size two pots). The soil moisture and nitrogen treatments were applied once each week. The plants were grown together for 20 weeks.

Above and below ground biomass was harvested for both species within each treatment. The above ground plant material was clipped 2 cm (.78 inch) above the soil surface and dried and weighed. The below ground biomass was removed from the pots, washed, dried and weighed.

The mean biomass values of RCG grown alone and RCG grown with SFB was analyzed utilizing a Three Way Analysis of Variance. As noted above, soil moisture, nitrogen and competition were the three factors involved and there were two levels for each factor. The above ground, below ground, and total biomass means were analyzed separately to determine exact impacts of the SFB competition.

10.6 *Phalaris arundinacea* Carbohydrate Reserves Objectives

10.6.1 Introduction

Perennial grasses use underground storage organs for absorption of water and nutrients, stabilization of the plant, storing nutrient reserves for winter survival, and for the initial growth in the spring and re-growth after grazing (Weinmann 1948; White 1972). This energy source is used for new growth until there is sufficient photosynthesis available for the respiration of the plant (White 1972). Many researchers agree that the total available carbohydrates (TAC) within the belowground biomass of a weedy perennial is the best way to gauge the fitness of the plant with regard to managing environmental conditions and control techniques by humans (Comes 1971).

Weinmann, 1947 clarifies the term total available carbohydrates to be those carbohydrates used by the plant “as a source of energy or as building material, either directly or indirectly after having been broken down by enzymes.” Total Nonstructural Carbohydrates (TNC) is considered to be those carbohydrates that are immediately available to the plant, mobilized for metabolism or translocated throughout the plant (Smith 1969). Smith (1969) prefers the term total nonstructural carbohydrates (TNC) as it is applicable for both plant and animal researchers.

10.6.2 *Phalaris arundinacea* carbohydrate storage

Several researchers have determined that the predominate polysaccharide reserves for reed canarygrass (RCG) are fructosans (Smith 1968, Reinhardt 2004). Smith (1968) specified that longchain fructosans prevail in RCG, however, many species that accumulate fructosans in this manner tend to contain short chain molecules during the time in the storage cycle where the TNC's are at their lowest point. Fructosans are fructose polymers found in two forms, inulins and levans, containing a terminal glucose residue and are water soluble (Smith 1969).

Reed canarygrass rhizomes predominately originate from buds at the nodes of other rhizomes below the soil surface. In a study from northern Ohio, new shoots for this species develop early in the season, around April and May in the mid-west, probably earlier here in the PNW, and in August until the end of the season. The shoots which developed early in the season are from rhizomes which developed late the prior year. Rhizomes which developed in mid season, such as May and June terminate into shoots that same season. New rhizome growth reached a maximum, double of any other month, in June of each year during this study as seen also by Evans and Ely (1935).

The rhizomes of RCG originate at a bud and grow outward, then turning upward to the surface producing an aboveground shoot. At this point, a new rhizome develops from a bud near the tip of the original rhizome or from a leaf axil and tends to grow in the same direction as the older one and a third rhizome also follows this same pattern. When comparing rhizome growth of five different species, one of which was RCG, Evans and Ely (1935) found that quackgrass was superior in the final diameter of the plant and rhizome length, showing the relationship between rhizome length and the area occupied by the plant. However, reed canarygrass was found to make up for less length by developing seven orders (number of branching events from the

original rhizome) of new rhizomes during which most of the other species only formed four orders of rhizomes (Evans and Ely 1935). This may also explain the substantial density of the rhizome mass of reed canarygrass.

Restoration of wetlands invaded by RCG is particularly problematic as this species is extremely difficult to eradicate or control. In addition to the highly competitive nature of RCG and other plant characteristics described in Chapter One, the predisposition of the rhizomes to persist with stored energy and produce new shoots after treatment makes control exceptionally arduous (Lyons 1998; Apfelbaum and Sams 1987; Lavergne and Molofsky 2004; Reinhardt 2004). The ability to store the nonstructural carbohydrates in the rhizomes also allows RCG to successfully overwinter and produce new tillers early the next season as well as continue productivity later in the season, which increases the competitiveness of this species (Lavergne and Molofsky 2004). This mat of rhizomes can also produce a sod layer up to 0.5 meters thick, making the establishment of native species challenging (Tu 2004). Additionally, the energy stored allows for the new tillers to actually lift and break through standard weed fabrics permitting continued growth of the plant from underneath.

Depleting the carbohydrate storage capacity of the RCG rhizomes is vital. Reducing the capability of the rhizomes to produce new plants after mechanical or chemical treatment would be advantageous for wetland restoration projects throughout western Washington. The objective of this project is to determine how long a land manager should cover RCG rhizomes with opaque material for complete depletion of the carbohydrate reserves before removing the material for planting or before planting within the material.

10.6.3 Carbohydrate reserve methods

Reed canarygrass rhizomes were analyzed for total nonstructural carbohydrate (TNC) reserves, or fructosans. Samples were taken during mid June of 2005 during anthesis, when the reserves are at their lowest point (Comes 1971; Reinhardt and Galatowitsch 2004). Forty-five rhizome samples of similar length and weight were randomly chosen and removed from the agriculture site.

Five gallon containers were placed in holes that had been excavated at the agriculture site, with the top of the containers level with the surrounding soil. The forty five rhizomes were placed within sterile potting soil in two gallon containers set within the five gallon containers with a root barrier placed at the bottom of the larger container so that any roots or rhizomes from potential growth could not escape. A thick, dense fabric was placed on top to inhibit any photosynthesis and stakes were used around the edges of the fabric. Fifteen samples were removed after three months and frozen. Another fifteen samples were removed after six months and the last fifteen after nine months and was stored in a frozen condition below 0° C.

A Fructan Assay Kit was obtained from Megazyme International Ireland Ltd., Wicklow, Ireland. This kit includes sucrose, fructanase, fructan control flour, sucrose control flour, and fructose standard solution. Additional chemicals required were ingredients for two buffers, a sodium maleate buffer and a sodium acetate buffer and several reagents; a PAHBAH reducing sugar assay reagent and alkaline borohydride (Megazyme Intl. 2004).

The samples were removed from the freezer, rinsed and dried at 70° C in a forced air oven at the Center for Urban Horticulture to a constant weight. The rhizomes were ground in a Wiley mill with a 0.5 mm (.019 inch) screen for analysis. The samples for each of the three time frames (3, 6, 9 months) were combined and 10 replicates were analyzed for each time frame. Randomly selected samples were used to calibrate the NIRS identifying fructosan specific wavelength spectra.

The Megazyme kit employs purified enzymes to hydrolyse sucrose, starch and fructans. The sucrase enzyme “hydrolyses sucrose but has negligible activity on I-kestose and other fructo-oligosaccharides.” The final reading solution is measured with the near-infrared spectrophotometer (NIRS) at the absorbance level of $\lambda = 409.64$ nm. The results were then calculated using the following equation (Megazyme International 2004):

$$C = A \times F \times 5 \times V \times 1.1/0.2 \times 100/W \times 1/1000 \times 162/180$$

where C is the fructan [% w/w], A is the sample absorbance minus the sample blank absorbance (read against reagent blank), F is a factor to convert absorbance values to μg of D-fructose ($54.5 \mu\text{g D-fructose}/(\text{absorbance for } 54.5 \mu\text{g D-fructose})$), V is the volume of extractant used [mL], W is the weight of sample extracted [mg], 5 is a factor to convert from 0.2 mL as assayed to 1.0 mL, the ratio 1.1/0.2 comes from the fact that 0.2 mL was taken from 1.1 mL of enzyme digest for analysis, the term 100/W is a factor to express fructan as a percentage of flour weight, 1/1000 is a units conversion factor from μg to mg, and 162/180 is a factor to convert from free fructose (D-fructose), as determined, to anhydrofructose (and anhydroglucose), as occurs in fructan. This analysis took place at the Center for Urban Horticulture, University of Washington.

10.7 Project Results

10.7.1 Field Project

The data shown in Figure 10-8 allows us to evaluate the effects of the treatments at all three sites together on the same plot. This plot reveals that all three treatments resulted in fewer returning RCG stems than what was established in the control plots as stem counts for the control plots are higher than for all three treatments at each site. The RCG barrier alone treatment was the least successful at reducing the returning RCG stem count at all three sites. The treatments involving the Hogfuel/Willow (HF/Willow) and the HF/RCG barrier were the most successful. The HF/Willow treatment was more successful at the agriculture site and the HF/RCG barrier treatment was more successful at the natural site. The HF/Willow and HF/RCG barrier treatments were not significantly different at the livestock site. Additionally, the livestock site had higher stem counts than the other two sites for the control and the other two treatments with the exception of the HF/RCG barrier treatment at the agriculture site.

A significant treatment and site interaction does exist ($p\text{-value} = .043$). As illustrated below in Figure 10-9, this interaction is due to the HF/RCG Barrier treatment obtaining a higher RCG

stem count at the agriculture site than at the other two sites. If the line were parallel there would not be a site/treatment interaction.

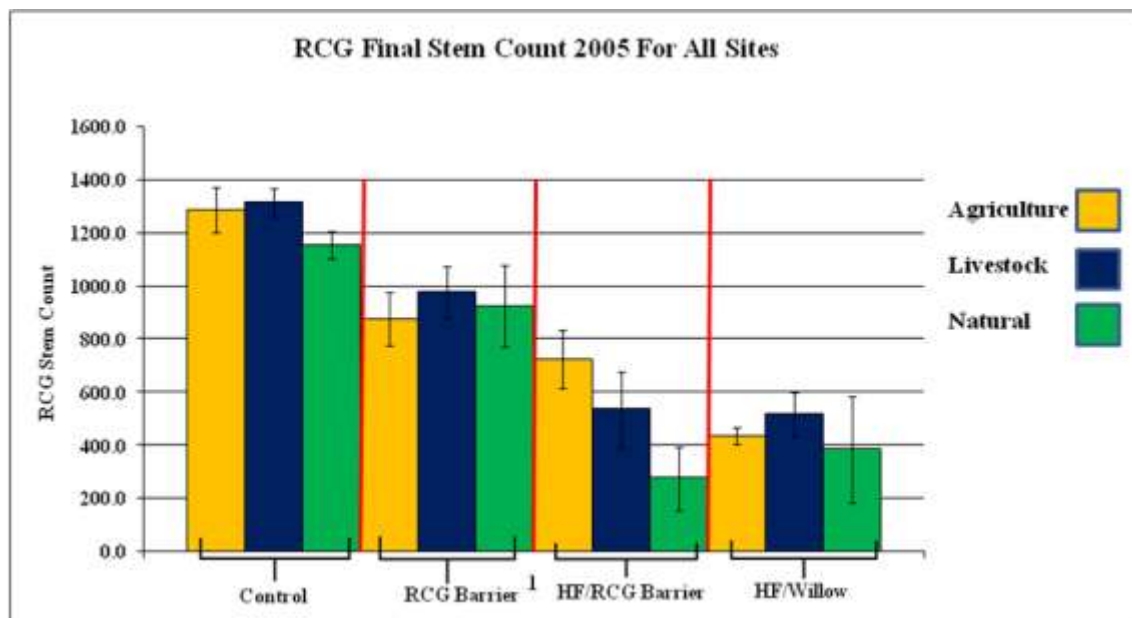


Figure 10-8. RCG final stem count from the three sites grouped by treatment.

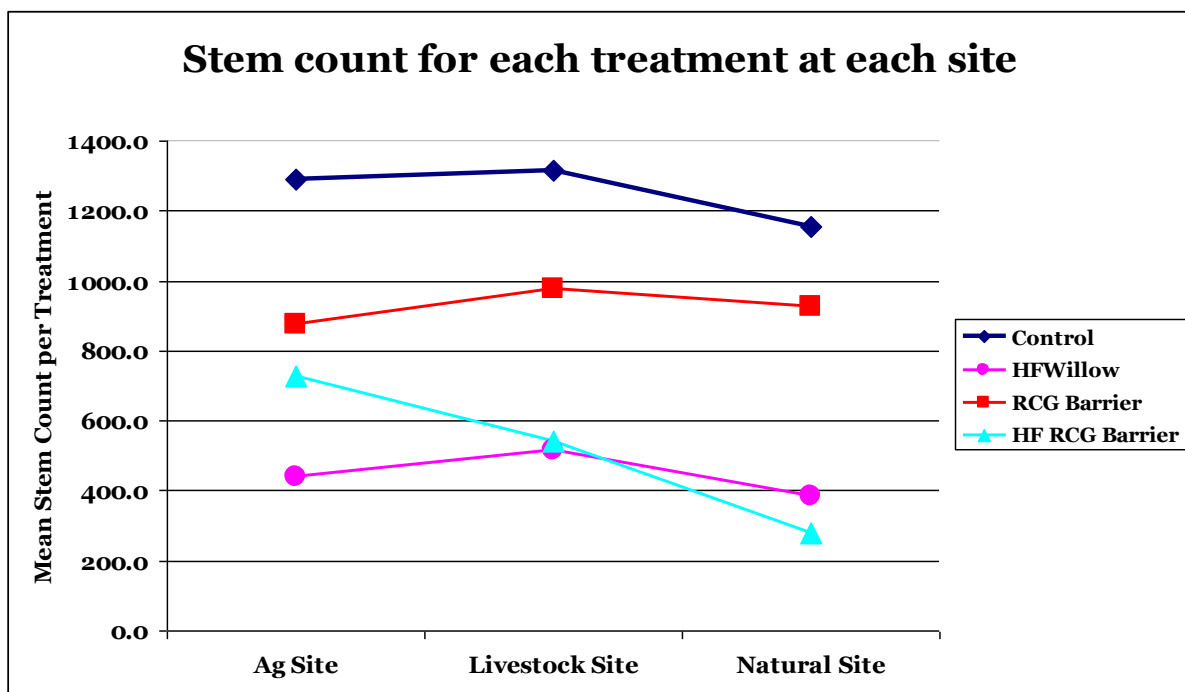


Figure 10-9. Stem count for each treatment at each site.

A Two-way Analysis of Variance (ANOVA) was used to examine the treatment main effect, site main effect and their interaction effect on the stem count. The ANOVA results are presented in Table 10-4.

Table 10-4. Two-way ANOVA results

	Df	Sum of Sq	Mean Sq	F Value	p
Treatment	3	3337438	1112479	101.74	0.000
Site	2	132895	66447	6.07	0.008
Treatment:Site	6	178229	29705	2.72	0.043
Residuals	20	218695	10935		

Examining the results of the treatments, each of the treatments were statistically significant when compared to the control RCG stem count return and when compared to the other treatments overall (p-value = .000).

Both treatment and site have a statistically significant effect on the stem count (p=0.000 for treatment and p=0.008 for site). Post hoc comparisons shown below in Table 10-5 illustrate the pair wise difference among all of the combinations of treatment and site. The Tukey HSD procedure was utilized to guarantee the overall alpha level of 0.05.

Figure 10-10 illustrates that the Hogfuel/willow treatment significantly reduced the returning RCG stem count when compared to each of the other two treatments at the agriculture site. A similar trend in reduction is shown in Figure 10-11 for the livestock site, although the difference between the Hogfuel/willow and the Hogfuel/RCG barrier is not statistically significant. For the natural site shown in Figure 10-12, the Hogfuel/RCG barrier stem count is actually less than the Hogfuel/willow treatment although not by a statistically significant amount.

When examining the results at the natural site, it is important to remember that the stem counts were from two replicates as replicate number one was completely flooded in late spring of 2004 after the watercourse channel moved during a major flood event. This site was also impacted by other flood events and beaver attacks on all of the woody species.

Table 10-5. Post-hoc multiple comparison results

	Comparison	Estimate	Lower Bound	Upper Bound	Sig¹
Agriculture Site	CON – HFWill	850	536	1160	*
	CON – HF/RCGbar	563	249	877	*
	CON – RCGbar	413	99.1	727	*
	HFWill – HF/RCGbar	-286	-600	27.5	
	HFWill – RCGbar	-437	-751	-123	*
	HF/RCGbar – RCGbar	-150	-464	164	
Livestock Site	CON – HFWill	798	484	1110	*
	CON – HF/RCGbar	775	461	1090	*
	CON – RCGbar	336	22.5	650	*
	HFWill – HF/RCGbar	-23.3	-337	291	
	HFWill – RCGbar	-462	-776	-148	*
	HF/RCGbar – RCGbar	-439	-753	-125	*
Natural Site	CON – HFWill	770	385	1150	*
	CON – HF/RCGbar	878	494	1260	*
	CON – RCGbar	231	-153	616	
	HFWill – HF/RCGbar	109	-275	493	
	HFWill – RCGbar	-538	-922	-154	*
	HF/RCGbar – RCGbar	-647	-1030	-263	*

Note¹ - * denotes significant at 0.05 level.

CON=control plot

HFWill=hogfuel willow

HF/RCGbar=hogfuel/reedcanarygrass barrier

RCGbar=reedcanarygrass barrier

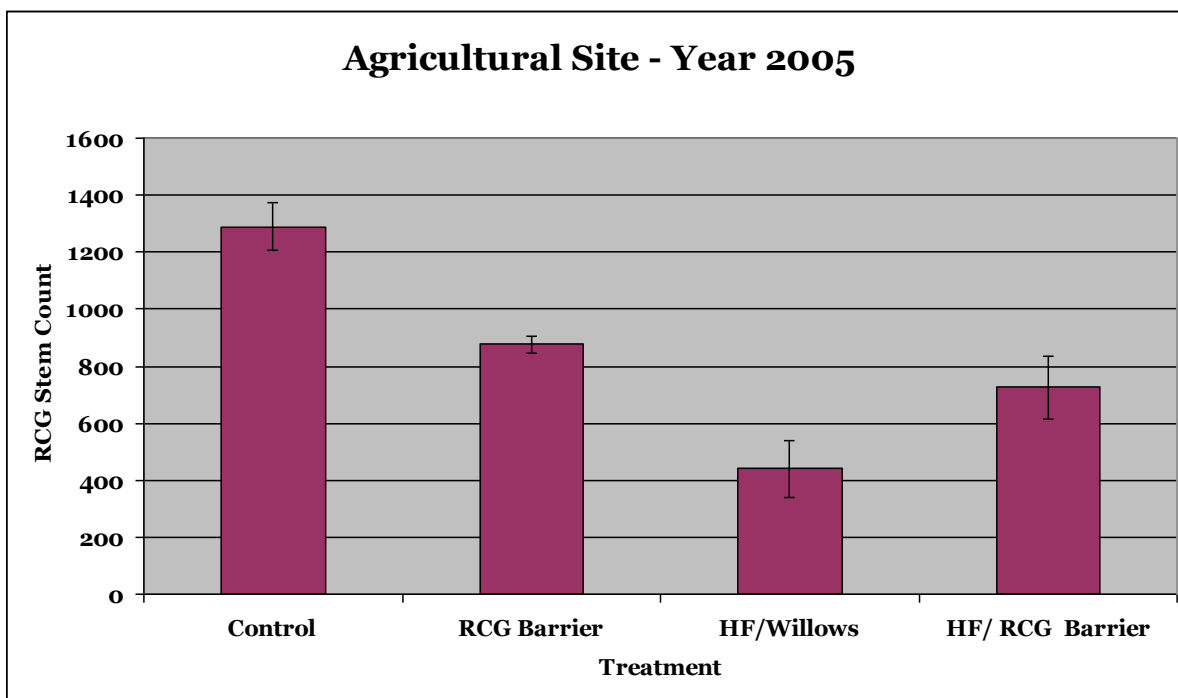


Figure 10-10. RCG stem count versus treatment methodology at the horticultural site

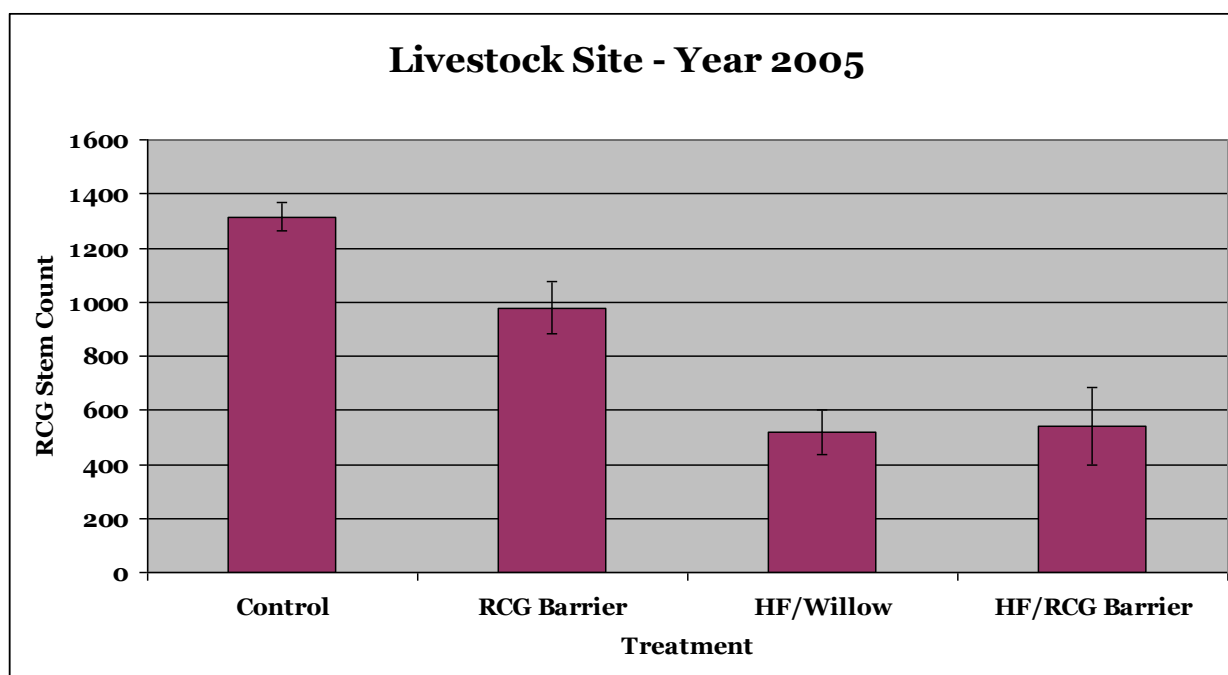


Figure 10-11. RCG stem count versus treatment methodology at the livestock site

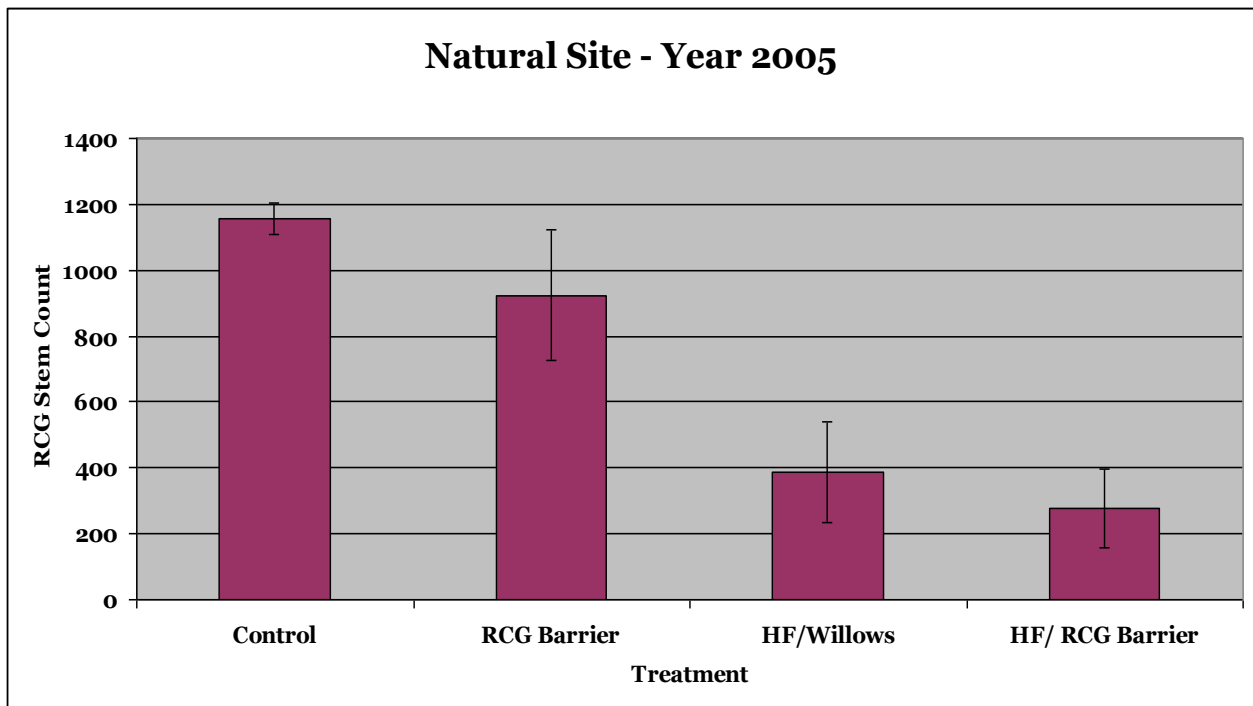
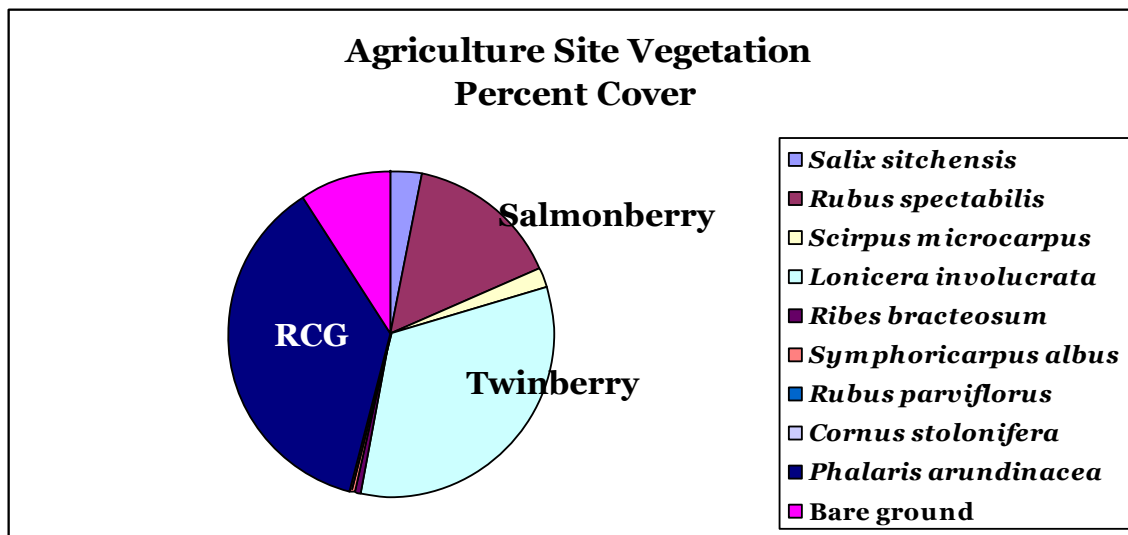


Figure 10-12. RCG stem count versus treatment methodology at the natural site

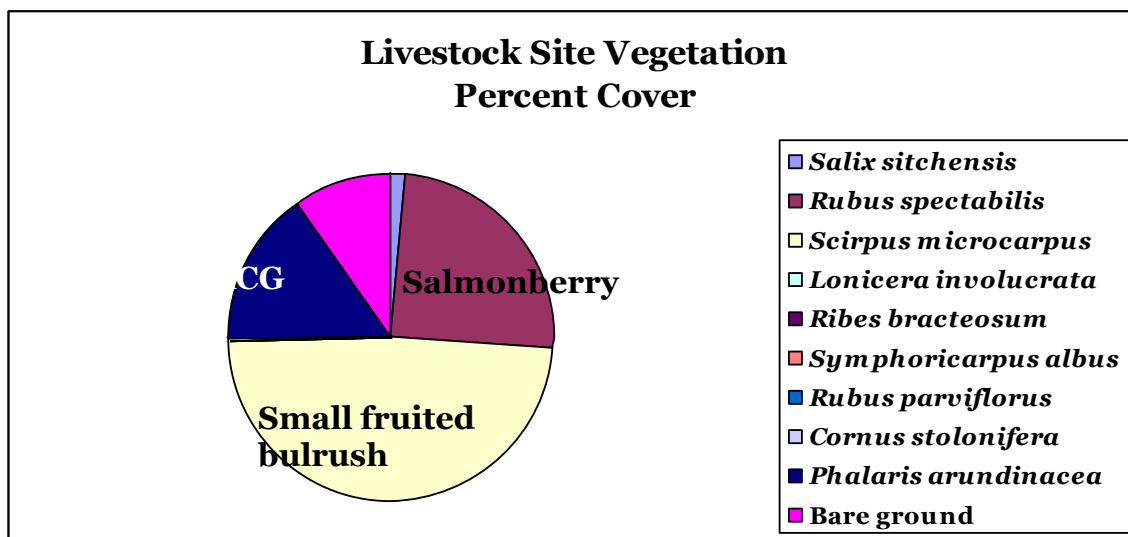
In addition to counting the returning RCG stems, the percentage of vegetative cover was also determined for each plot at each of the sites. Figure 10-13, Figure 10-14, and Figure 10-15 illustrate the vegetative cover percentages for the horticultural, livestock, and natural site, respectively. Due to high variation within each plot, the six (out of 12) quadrates closest to the watercourse were utilized for the agriculture and livestock sites. These charts and affiliated pictures reveal which species used in this study not only survived the harsh conditions of competing with established RCG, high variations in water levels, flooding, beaver attacks and the willow borer, but also which species thrived.



Hogfuel/RCG barrier treatment
Average of 3 replicates / 6 quadrates closest to the watercourse



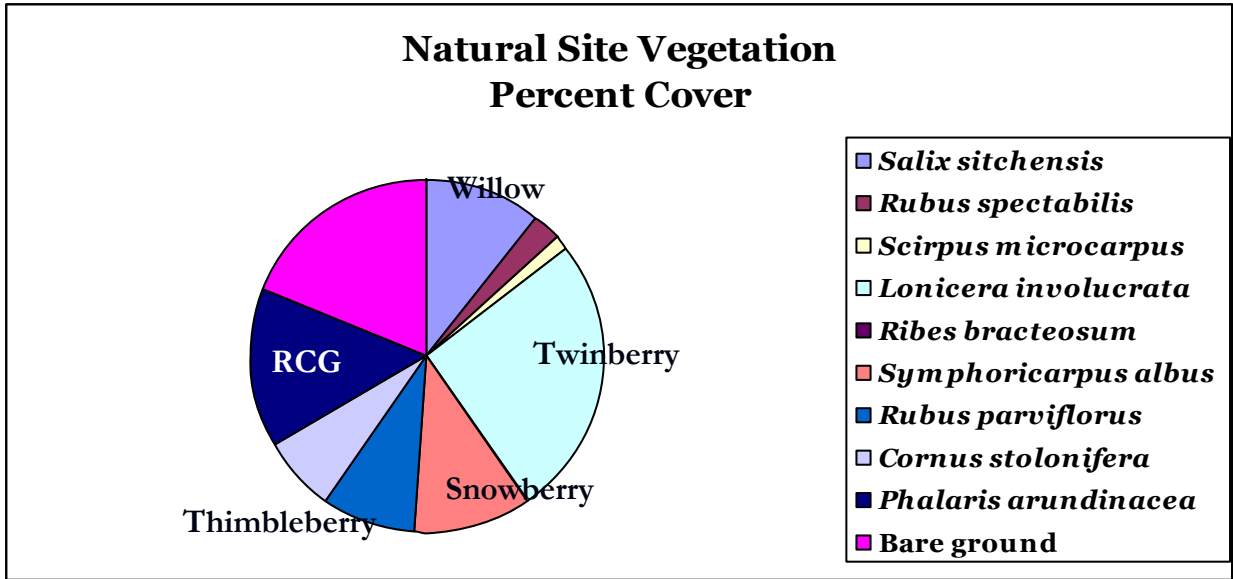
Figure 10-13. Vegetative cover at horticultural site



Hogfuel/RCG Barrier treatment
Average of 3 replicates from the 6 quadrates closest to the watercourse



Figure 10-14. Vegetative cover at livestock site



Hogfuel/RCG barrier treatment
Average of 3 replicates



Figure 10-15. Vegetative cover at natural site

10.7.2 Allelopathy Test Results

Red Cedar Hogfuel was tested for allelopathic tendencies on lettuce seed germination, seedling growth and RCG rhizome regrowth. Seeds, seedlings and rhizomes were watered with the hogfuel tea for those in the HF treatment and watered with fresh water for the control treatment. Red Cedar Hogfuel was inundated with water for 72 hours to make the hogfuel tea. Ten replicates of five lettuce seeds were placed on filter paper and watered daily with hogfuel tea or water for five weeks. The results are illustrated below in Figure 10-16 (seed germination), Figure 10-17 (seed growth), and Figure 10-18 (RCG rhizome regrowth).

The results indicate that the Red Cedar Hogfuel is allelopathic for lettuce seed germination and growth by significantly reducing the number of germinating seeds (T-test, $df = 9$, $p = 0.005$) and radicle length (T-test, $df = 9$, $p = 0.000$). The Red Cedar hogfuel tea also significantly reduced the RCG rhizome regrowth when comparing the stem count of the rhizomes grown with hogfuel tea versus those grown with water, with a p-value of .05 (t-Test for a paired two sample for means).

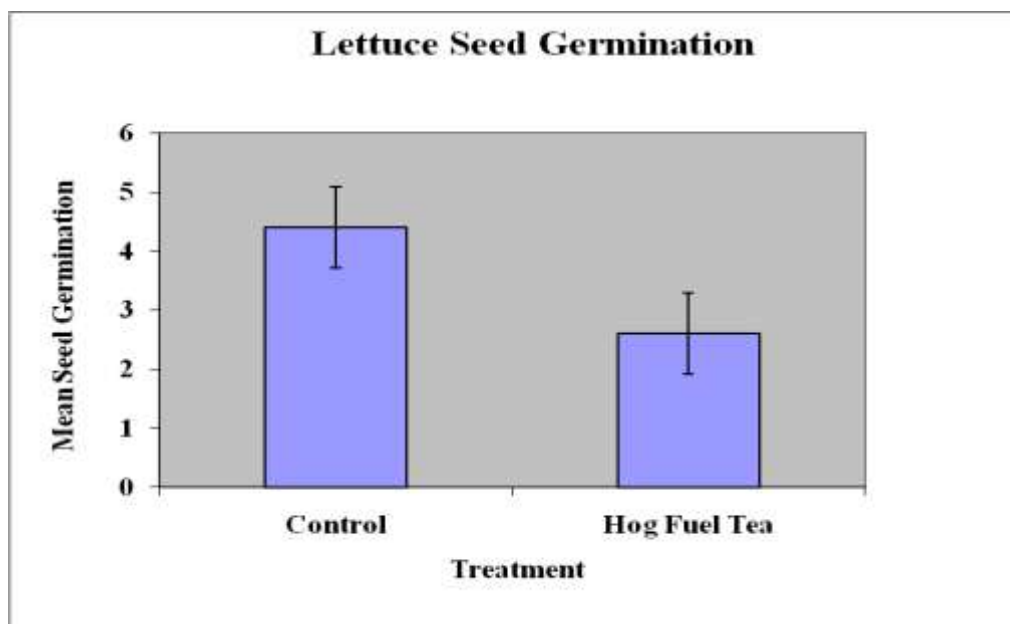


Figure 10-16. Germination of lettuce seeds using fresh water versus hogfuel tea

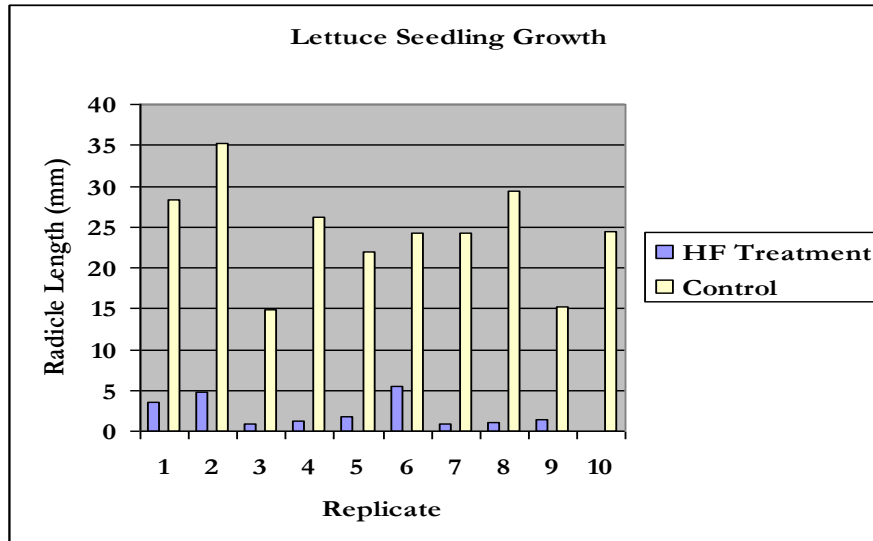


Figure 10-17. Radicle length of lettuce using fresh water versus hogfuel tea

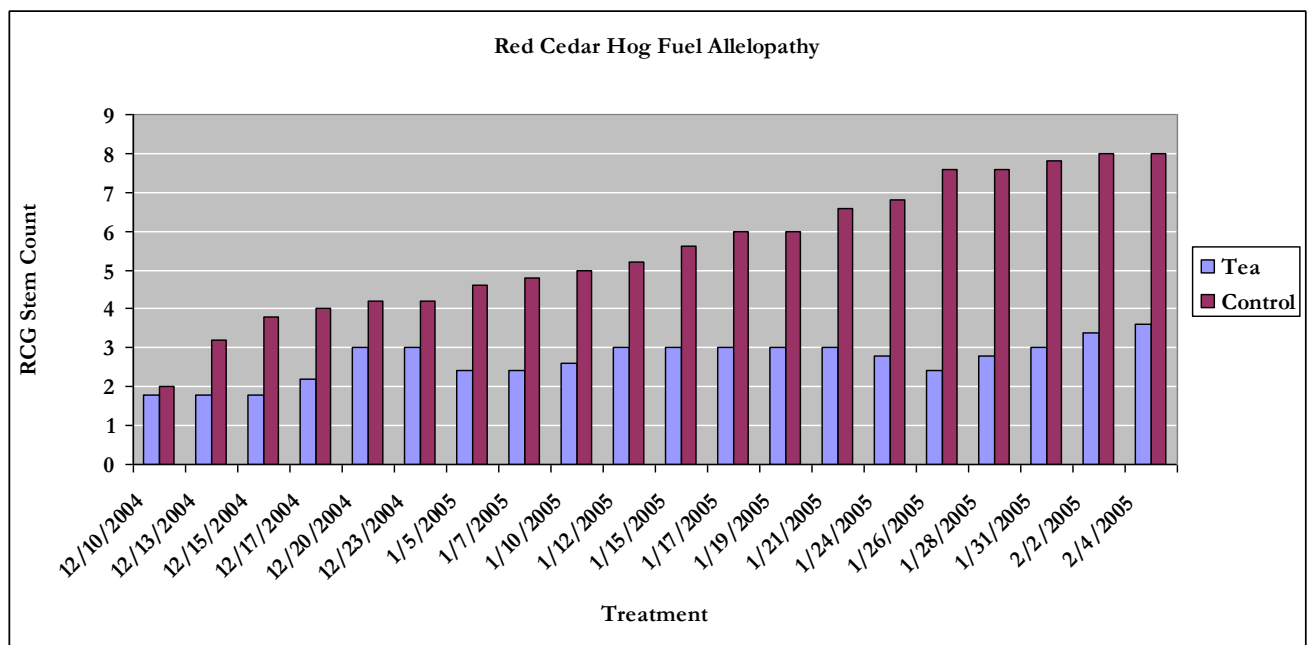
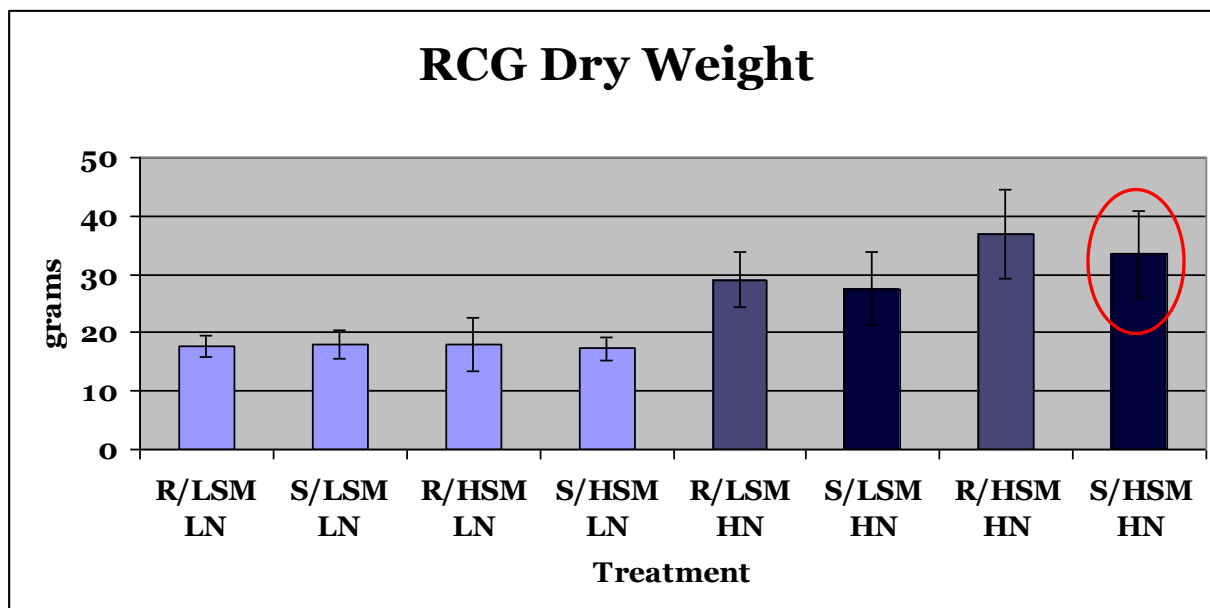


Figure 10-18. RCG rhizome regrowth using fresh water versus hogfuel tea

10.7.3 Competition Experiment Results

As expected, both RCG and SFB species responded favorably to the high nitrogen treatments. The RCG plants within the low nitrogen treatments did not respond differently to high or low soil moisture treatments or to inter- versus intra- specific competition. Conversely, the RCG plants exposed to the high nitrogen treatments did exhibit greater biomass in the high soil moisture treatments. Although not significant, within the high nitrogen/high soil moisture treatments, the RCG grown with inter-specific competition (with SFB), showed evidence of lower total plant biomass (above and below ground) overall than when grown with intra-specific competition (with another RCG plant) (t-test, $p = .30$). Figure 10-19 graphically illustrates the comparisons.

While examining the above and below ground biomass, it was observed that the RCG consistently allocated more resources to above ground biomass generation within the high nitrogen treatments (see Figure 10-20) while the SFB consistently allocated more resources to below ground biomass generation within all treatments (Figure 10-21). In fact, when looking at Figure 10-20, both above and below ground RCG biomass are reduced when grown with SFB in high nitrogen environments. Within the HSM/HN treatment, SFB reduced the RCG below ground biomass more than above ground biomass. It seems plausible that the SFB is utilizing excessive below ground biomass when competing with other species.



R = RCG vs. RCG
S = RCG vs. SFB

LSM = Low Soil Moisture
HSM = High Soil Moisture
LN = Low Nitrogen
HN = High Nitrogen

Figure 10-19. Total RCG dry weight grown with *Scirpus microcarpus*

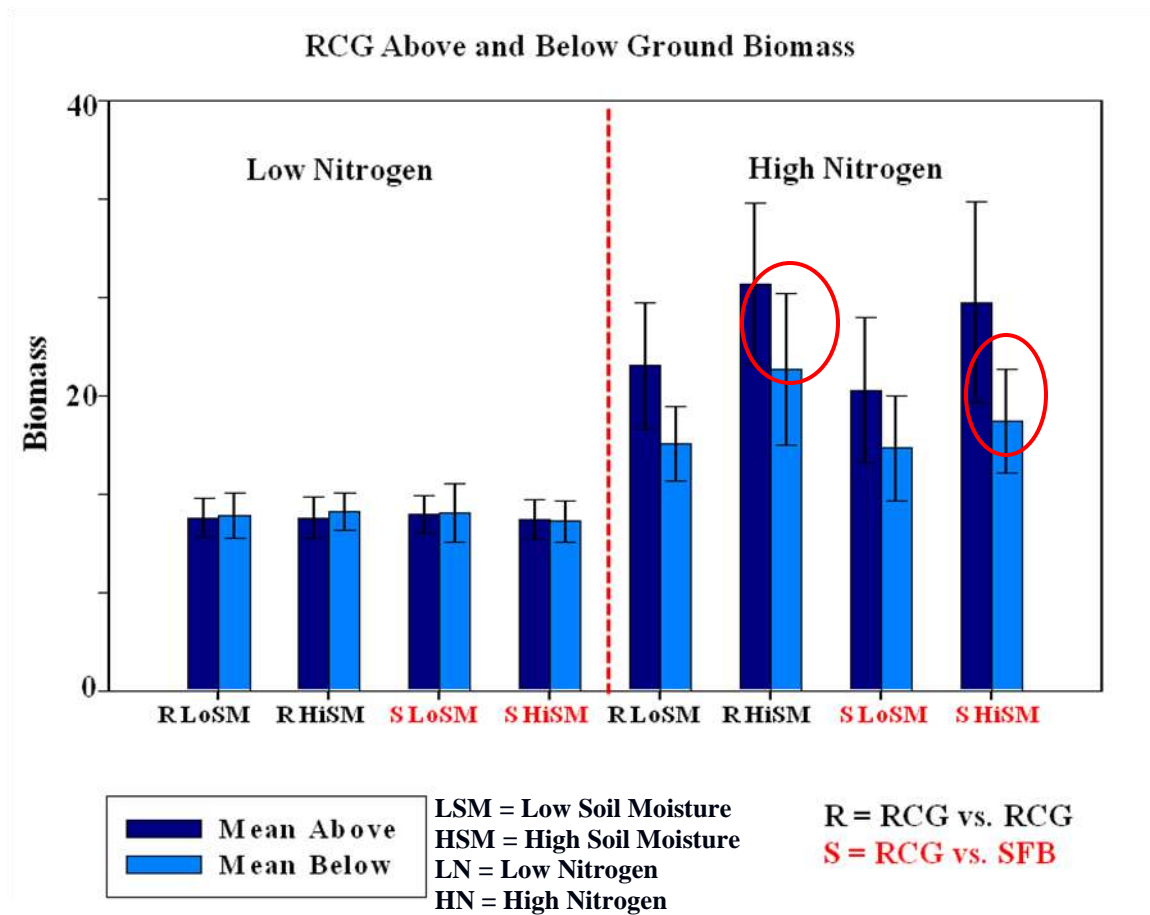


Figure 10-20. RCG above and below ground dry weight for each treatment

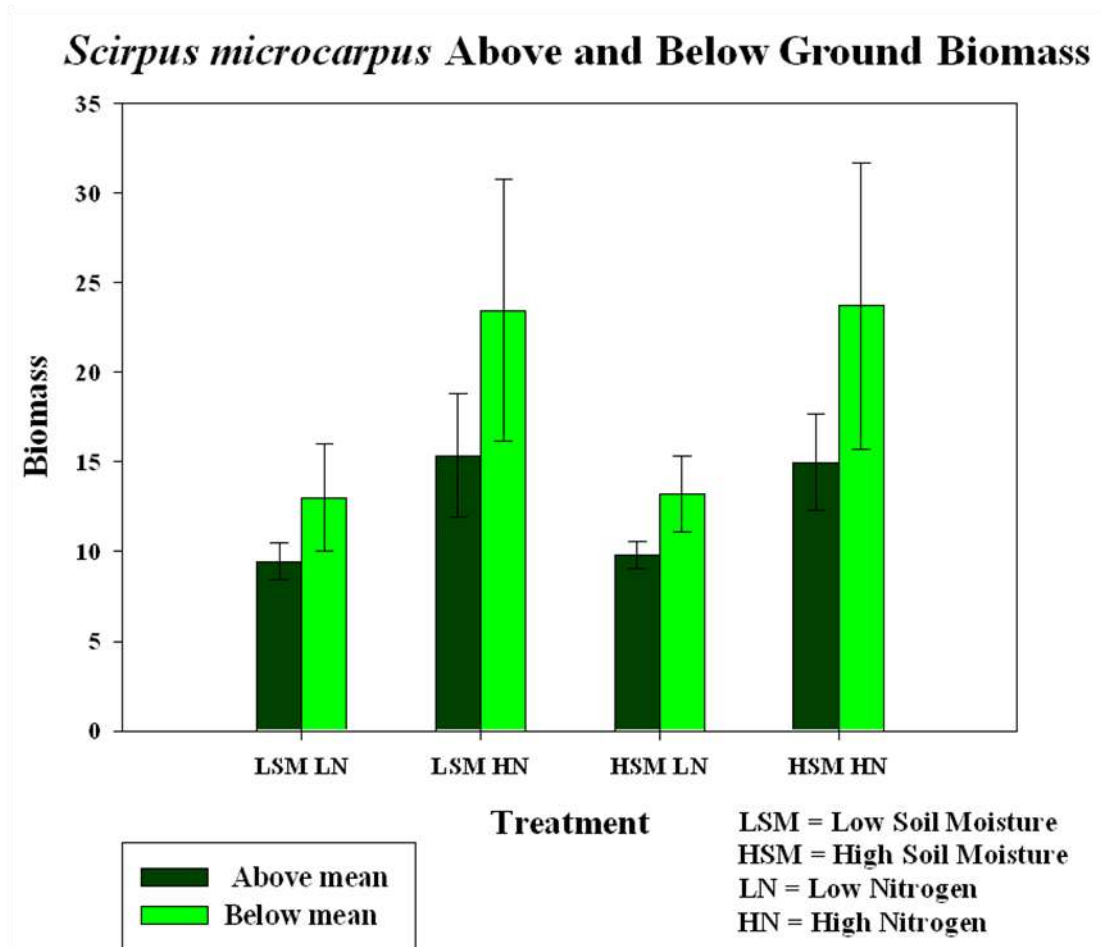


Figure 10-21. SFB above and below ground dry weight for each treatment

10.7.4 Carbohydrate reserve project results

The results indicate an extensive variation of the fructosan levels within the rhizomes which were covered for three months among ten replicates analyzed. Five replicates were able to be analyzed for the six month batch and three replicates for the nine month batch. The average percent of fructosans for those rhizomes covered for three months is 2.25%, for the six month rhizomes, 1.65% and for the nine month rhizomes, 0.773% (Figure 10-22).

Due to the variability among individuals, the R^2 value of the regression of fructosan levels over time is very low ($R^2 = 0.1262$). The scatter is clearly shown in Figure 10-23.

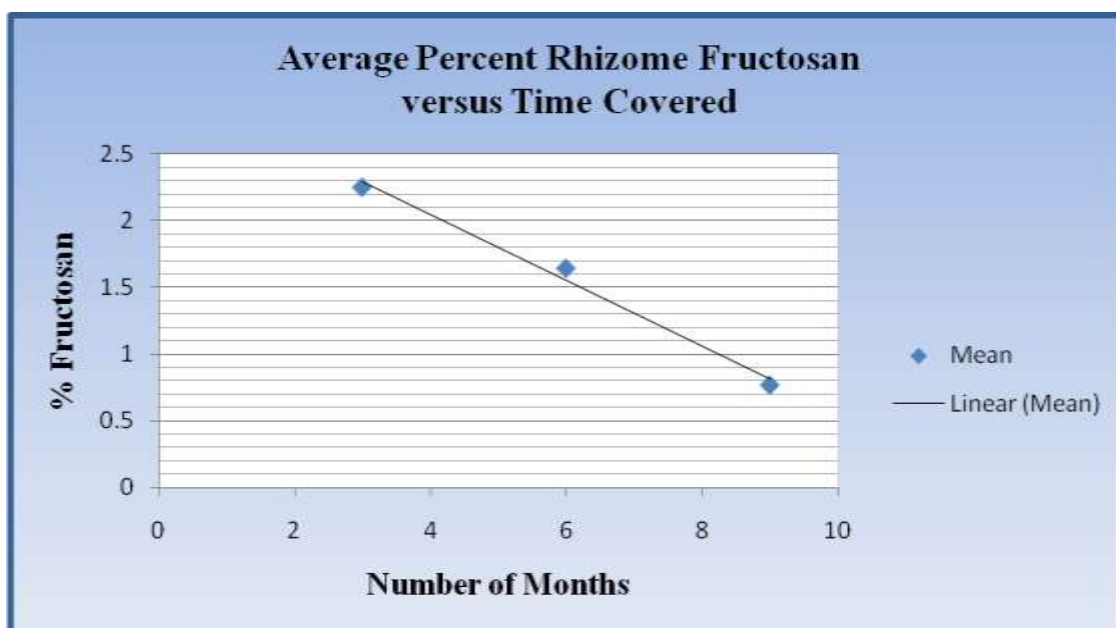


Figure 10-22. Average Percent Fructosan level for three, six and nine months

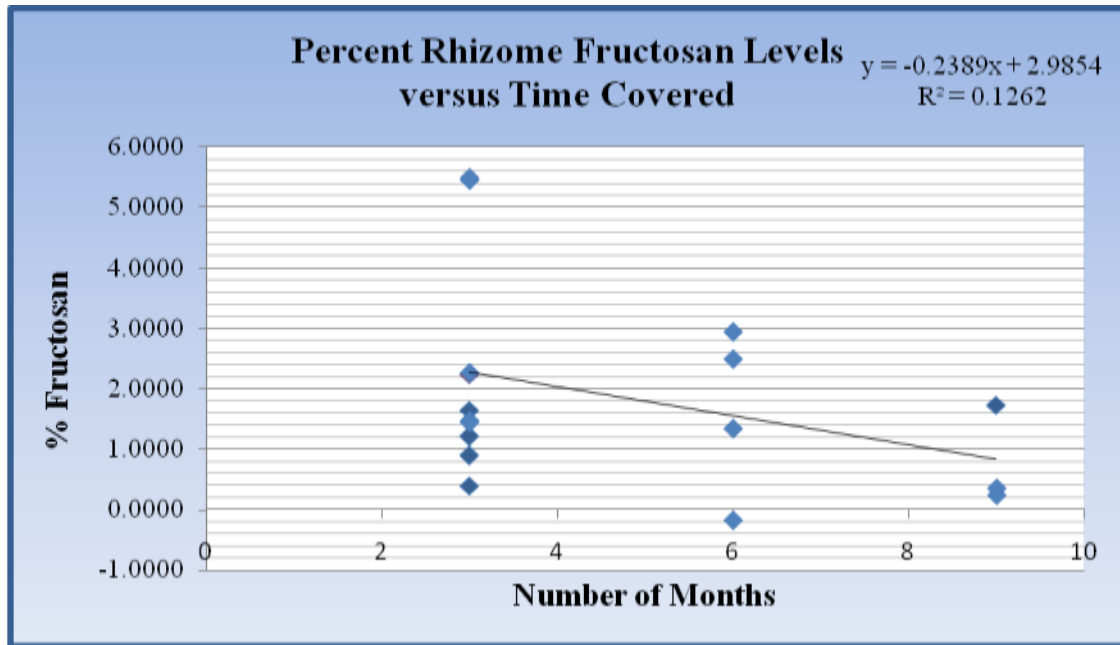


Figure 10-23. Percent Rhizome Fructosan levels for three, six and nine months

One rhizome sample within the six month batch was almost black after being removed from the field, while every other rhizome was tan or tanish pink. Although not characteristic of the other rhizomes, the sample was analyzed out of curiosity. It did indeed produce a skewed fructosan result with a negative number of -0.18. After removing this sample, as it was obviously a dead rhizome that had desiccated in the field, the R value decreased further to a 0.11 (Figure 10-24). Also, after removing the dead rhizome, the means of the three and six month samples become equal at 2.25 % compared to the 0.77 % for nine month sample.

Log transforming the data does not improve the R value ($R^2 = 0.20$) indicating that the variability is too high and there are not enough data to show a significant non-linear fit of the data to the line. However, log transforming the data for a Single Factor Analysis of Variance test (Single Factor ANOVA, $df = 2$, $p = 0.08$) indicated the lack of a difference between the three month mean and the six month mean. Yet, there is enough of a difference between the three and six month mean when compared to the nine month mean to provide a p-value that is marginally significant (Figure 10-25).

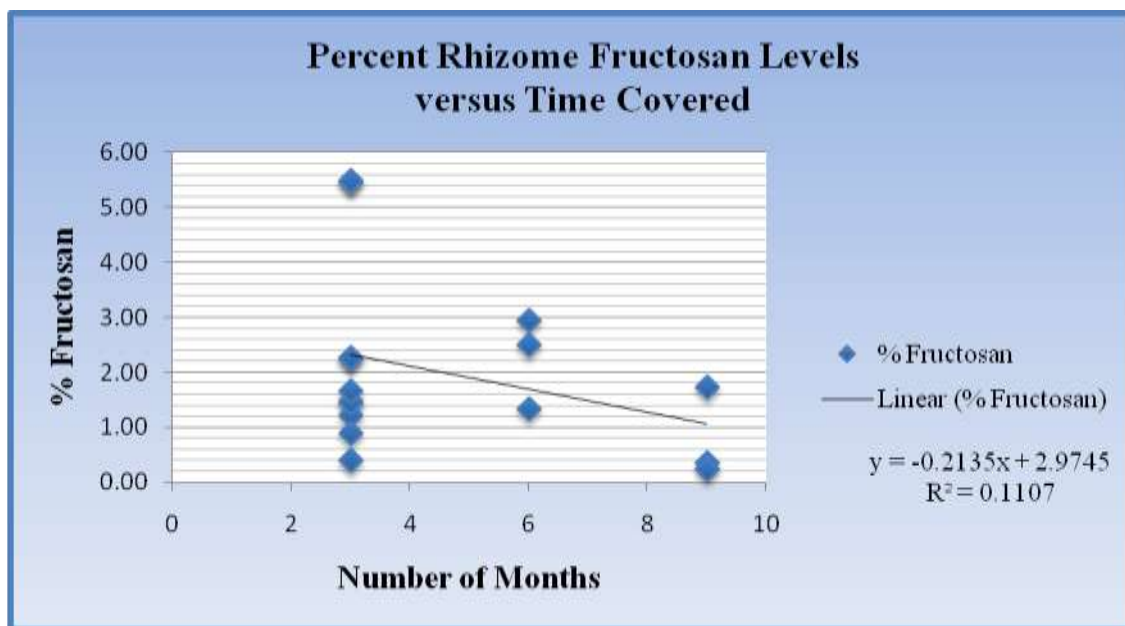


Figure 10-24. Percent Rhizome Fructosan levels for three, six and nine months after removing the dead rhizome (negative) data point.

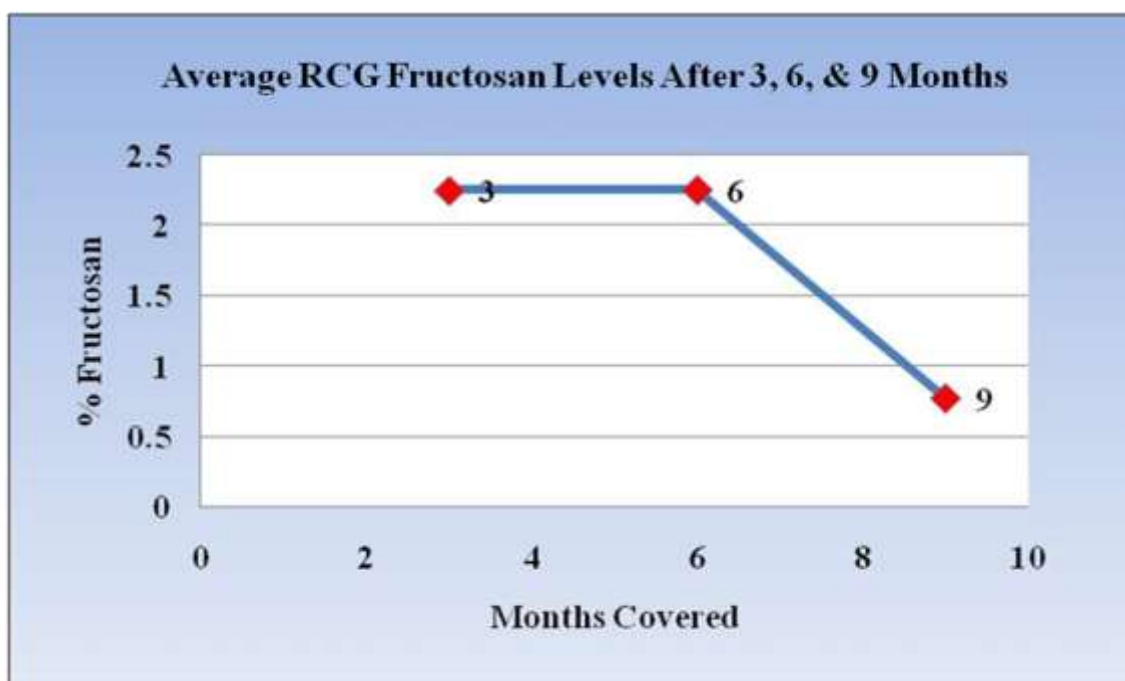


Figure 10-25. Mean RCG Fructosan levels after removing negative data point.

10.8 Discussion and Recommendations

10.8.1 Field Experiment

Both the treatment and the site affected RCG stem count (RCG re-growth). The treatment effect varied by site for one treatment alternative (the HF/RCG Barrier treatment at the Agriculture site). The post hoc comparisons quantified the differences among treatments at each site. These tests indicate that the control stem counts were significantly higher than all three treatments at all sites, except at the livestock site, where control count is not significantly different than the RCG Barrier alone treatment.

However, as previously shown in Figure 10-8, the HF/Willow treatment has lower stem count numbers overall. This may be due to the unexpected positive effects provided by the compost added to the RCG Barrier and HF/RCG Barrier treatment for planting within. The compost most likely provided additional moisture and possibly nitrogen for the RCG.

One of the objectives of this research project was to provide maximum shade and preferred invertebrates for salmonids, whether to benefit salmonids within the watercourse itself or downstream within the main channel into which the watercourse flows. The HF/Willow treatment alone would not be able to adequately meet this objective. Past research has shown that RCG responds negatively to numerous canopy layers (Lindig-Cisneros and Zedler 2001, Maurer and Zedler 2002). Stem count results from my mixed canopy layer plots echo those results. Additionally, a more diverse herbaceous and woody plant species assemblage would allow for a more diverse invertebrate community (Allan et al. 2003).

The hogfuel added, densely planted willow treatment and hogfuel added and the densely planted multi-canopy treatment (RCG barrier) reduced the returning RCG biomass by 64% and 56% respectively compared to the control RCG stem counts. The RCG barrier alone treatment was different than the controls and therefore, adding the hogfuel delivered the additional weight and solar radiation reduction necessary to suppress RCG re-growth. The red cedar hogfuel did show allelopathic tendencies in the greenhouse, however whether allelopathy played a role in the reduction of the returning RCG count in the field was not tested.

Based on this work, our recommendation for controlling RCG and restoring agricultural watercourses would be the Hogfuel/RCG Barrier treatment with the multiple species plantings. Providing multiple canopy layers would allow for lower light levels and the additional species (*Rubus spectabilis*, *Vaccinium ovatum* and *Ribes bracteosum*) would assist in providing preferred prey for salmonids.

Salix sp. generally do not leaf out until mid to late spring (~mid April) while RCG generally begins growth in January or February in the PNW. If other species are utilized within the restoration/replanting, there is a greater chance for a few of the species to leaf out earlier in the spring (for example, the *Scirpus microcarpus*, *Rubus spectabilis* and *Lonicera involucrata*) providing some shade to the early returning RCG and supplementary shade for the water course. The use of conifers may also be desirable but were inappropriate for this study due to the height

requirement of the Farm Preservation Program (FPP) program in King County under which this land operates.

With the Hogfuel/Willow treatment, the success of the restoration could be heavily impacted by the poplar and willow borer (*Cryptorhynchus lapathi*), an introduced species now ubiquitously established throughout western Washington. This weevil attacked the Agriculture and Livestock sites, at the end of the 2005 season and destroyed up to 80% of the willows at both sites (See Figure 10-26). The damaged willows were cut off, removed from the premises and destroyed so that the larvae inside would not mature. Many of the willows recovered from the rootstock, however, the shade supplied for RCG control was reduced dramatically and would not recover for at least 2-3 years.

Placing woodchips/mulch within wetland and/or riparian settings, even for restoration projects, is considered the filling of a wetland and the project is therefore subject to inspection by the US Army Corps of Engineers. The Corps will evaluate the project, and unless some aspect of the filling does not comply with the Endangered Species Act, the Corps will be able to allow the project under the Nation Wide Permit 27 (Bennett, pers. comm. 2008). Using red cedar hogfuel for RCG suppression was successful in this study and is recommended for use due to the weight, nitrogen reduction and potential allelopathic tendencies.



Figure 10-26. An adult willow borer (*Cryptorhynchus lapathi*) on an impacted willow stem at the livestock site

A study published after the red cedar hogfuel was tested in this research project also indicated inhibition of germination and suppression of hypocotyls and radicle growth of lettuce seeds and seedlings. Five species of trees from the southeast United States were tested. The southern red

cedar (*Juniperus silicicola*) also significantly inhibited the growth of a common weed in the southeast, *Desmodium tortuosum* DC (florida beggarweed) when compared to a gravel mulch and control (Rathinasabapathi et al. 2005).

Finally, if financially feasible, a thicker fabric should be used in the future in place of the burlap fabric for either of the treatments described above. A thick, compact, heavy, and fully biodegradable fabric is in the process of being developed for weed control within restoration projects but is not publicly available at this time.

It was obvious that some species were superior in their ability to survive, compete and thrive within the harsh conditions in which they were planted. A list of recommended species from the species that were employed in this project include: *Scirpus microcarpus*, *Rubus spectabilis*, *Lonicera involucrata*, *Salix sitchensis*, *Cornus sericea*, and *Rubus parviflorus*.

10.8.2 Competition Experiment

Both species in this study responded negatively to the low nitrogen treatments, whether grown with high or low soil moisture or with inter or intra specific competition. SFB did show slightly higher biomass levels under low nitrogen/low soil moisture treatments, but did not suppress RCG under these conditions any more than the RCG plant grown with intra-specific competition.

While looking at competition intensity and asymmetry, Johansson and Keddy (1991) found that the intensity of competition increases with species that are morphologically more similar and similar individuals have more symmetrical interactions. However, the authors also state that they believe their results may support the theory of co-existence proposed by Aarssen (1983) and Keddy and Shipley (1989).

This theory is stated as: “similar species will coexist because inter-specific competition is approximately equal to intra-specific competition, thereby weakening differential inter-specific interactions, which lead to exclusion” (Johansson and Keddy 1991). Perhaps, the competitive interactions between SFB and RCG are overly symmetrical and therefore, the theory that this weakens inter-specific interactions is accurate in this situation. If this is the case, using SFB to outcompete RCG may only be successful in highly specific situations, such as in sites with very high nitrogen and direct sunlight, as seen in the conditions at the livestock site initially. A total of 66.08 mg (0.02 oz) of nitrogen was used for the high nitrogen treatment based on the soil samples taken from the livestock site. However, this site may very well receive much higher pulses of nitrogen during rain events or possibly when the horse waste is not removed before a rain event. Higher nitrogen levels may have changed the results of the experiment and would be an interesting future study.

Gaudet and Keddy (1988) found that biomass, especially above-ground biomass, was determined to be the plant trait that was most strongly correlated ($r^2 = 0.75$) with competitive ability and the suppression of the phytometer used (*Lythrum salicaria*). Below-ground biomass, plant height and canopy area (cm^2) were also highly correlated, but not to the same extent (Gaudet and Keddy 1988). The authors theorize that when species with similar biomass levels are co-occurring, other factors such as height or other life history or morphological variables determine the

outcome of the competitive interaction (Gaudet and Keddy 1988). Again, possibly the two species, SFB and RCG are too similar in height and life history traits, and therefore, may only co-exist in most conditions.

SFB consistently produced more below-ground biomass over all treatments and less above ground biomass over all treatments than RCG. RCG did the opposite, at least in the high nitrogen treatments, producing more above-ground biomass than below-ground. Conceivably, the limitation of the pots in the greenhouse did not allow the SFB to produce sufficient below ground biomass to support additional growth above ground to effectively compete with RCG as this species did in the field for the first two seasons.

Small fruited bulrush competition impacted the RCG total plant biomass within the high nitrogen/high soil moisture treatment, although the difference was not statistically significant. These results also contradict a study by Green and Galatowitsch (2002) where total shoot and root biomass of the native community was suppressed by RCG, at all three levels of nitrogen used.

The SFB planted at the Horticultural site thrived and competed effectively with RCG, especially in the first season of this experiment, (Figure 10-27A) and even more so at the Livestock site during the 2005 season, (Figure 10-27B and Figure 10-28). These observations prompted the controlled competition experiment in the greenhouse. However, after several years, the shade from the surrounding vegetation and RCG on the other side of the bank seemed to negatively impact the SFB.

Even with the contradictory results, SFB can be recommended in certain re-vegetation situations. The livestock site received higher nitrogen levels than what was found at the other two sites and was the only site where the plots were facing south, getting direct, full sunlight throughout the day. Since SFB is a shade intolerant species, only using this species for restoring watercourses in situations where this emergent would be able to get full sunlight within high nitrogen environments is recommended.

Additionally, herbaceous wetlands (also referred to as Palustrine emergent wetlands by Cowardin et al. (1979)) are in serious decline nationally and within the Pacific Northwest. Although protected federally by the Section 404 of the Clean Water Act and in Washington State by the Shoreline Management Act and State Water Pollution Control Act (Granger et al. 2005), emergent wetlands have been extensively filled and drained for development, farming and for livestock. Excessive loss of acreage by non-native species invasions adds to this loss of acreage. Restoring impacted wetlands with woody vegetation in order to control exotics only exacerbates the loss of herbaceous wetlands by creating forested wetlands in their place. Adding emergent species to the re-vegetation of these agricultural watercourses would allow for the intended diversity one could obtain from multiple canopy layer situations.

A.



B.



Figure 10-27. SFB planted at the agriculture (A) and livestock (B) sites

A.



B.



Figure 10-28. SFB planted at the livestock site

10.8.3 Carbohydrate depletion experiment

One six month sample, replicate number two was considered an outlier with a value of 18.16% fructosans and was removed as it meets the standards of outliers for regressions, with eleven standard deviations above the mean. When examining the raw data for this sample, the sample absorbance was 1.5089% and 1.4635% which was much higher than every other sample absorbance in this experiment, ranging from 0.001 to 0.6 percent. All of the other rhizome

samples that were analyzed in the same batch as this sample, with the same fructose and sucrose cellulose and 4D-Fructan standards, were normal.

Reinhardt (2004) analyzed RCG rhizomes every two weeks throughout the growing season in Minnesota to determine the seasonal variation and the most favorable time for herbicide application. As with many perennial species, the pattern of RCG reserves illustrated a decrease early in the season as the new shoots developed, followed by a leveling off in reserves as the plant matured and increased photosynthesis levels. Each year showed a marked decrease in reserves in early July at which point the plants were flowering and setting seed. At the end of July, the reserves began to increase until the end of the season. All three years strongly varied in the levels of carbohydrate content for Reinhardt's study, yet the carbohydrate levels followed a similar pattern. The rhizome content at flowering was estimated to be ~5% for the year 2000, ~33% for the year 2001, and ~17% for the year 2002 (Reinhardt 2004).

Comes (1971) also found the lowest seasonal carbohydrate level for RCG took place at the time of anthesis, increasing gradually in the fall. While looking at the TAC levels in the roots and rhizomes of RCG for plants treated with herbicide versus plants that were not treated, the untreated plants averaged between 26 and 33% of the total dry matter. It should be noted that this researcher used a different extraction method, sulfuric acid solution and it is therefore, difficult to compare the TAC levels with the levels that were obtained.

Although a decreasing trend in fructosan levels was observed over time, there was no significant difference between the three time periods that the rhizomes were covered. However, when averaging the data points for each of the three time frames, there is a significant drop in fructosan levels after the six month time frame from just over two percent to just under one percent for those rhizomes covered for nine months. The rhizome carbohydrate levels seem to persevere through the first six month period but after that time frame, when removed from the parent clone, the fructosan levels begin to drop consistently.

RCG rhizome health diminished as time separated from the parent clone passed from three months to nine months. The number of rhizomes found in the pots decreased over time, and the size of many of the rhizomes found dwindled from ~ten cm (3.9 inches) long with several nodes to some less than half of the original size (Figure 10-29).

The degradation of the rhizomes once separated from the clone indicates that tilling the rhizomes seems to weaken their ability to survive over time, especially when keeping the rhizomes from exposure to solar radiation. The reduction in rhizome numbers and size may indicate that the wounds caused by breaking apart the rhizome mat may permit entry of microorganisms which in turn could instigate decay of the rhizome. This type of infection is well known with wounds in the bark of trees potentially causing the death of the individual (Harris et al. 1999).

Additionally, the propensity of a rhizome fragment to degrade without the connection to a parental clone may signify that the connections are important during periods of stress. The connection would allow for the rhizome or ramet to draw from a larger resource supply permitting endurance rather than disintegration as with stressed isolated individuals (Tomasko and Dawes 1989). When examining the TNC levels in grazed and un-grazed *Carex lyngbyei* by

Canada geese, Crandell (2001) did not find a significant difference between the samples. She noted that overall belowground biomass may be a better indicator of fitness than TNC concentrations. In other words, the sum of carbohydrate reserves available to the RCG clone from the entire rhizome biomass may be more significant than the concentration of reserves. If this is accurate, removing the rhizomes from the parent clone and reducing the size of the rhizome material is paramount in winning the battle with RCG domination due to rhizome re-growth after typical control methods such as mowing or herbicide treatments.

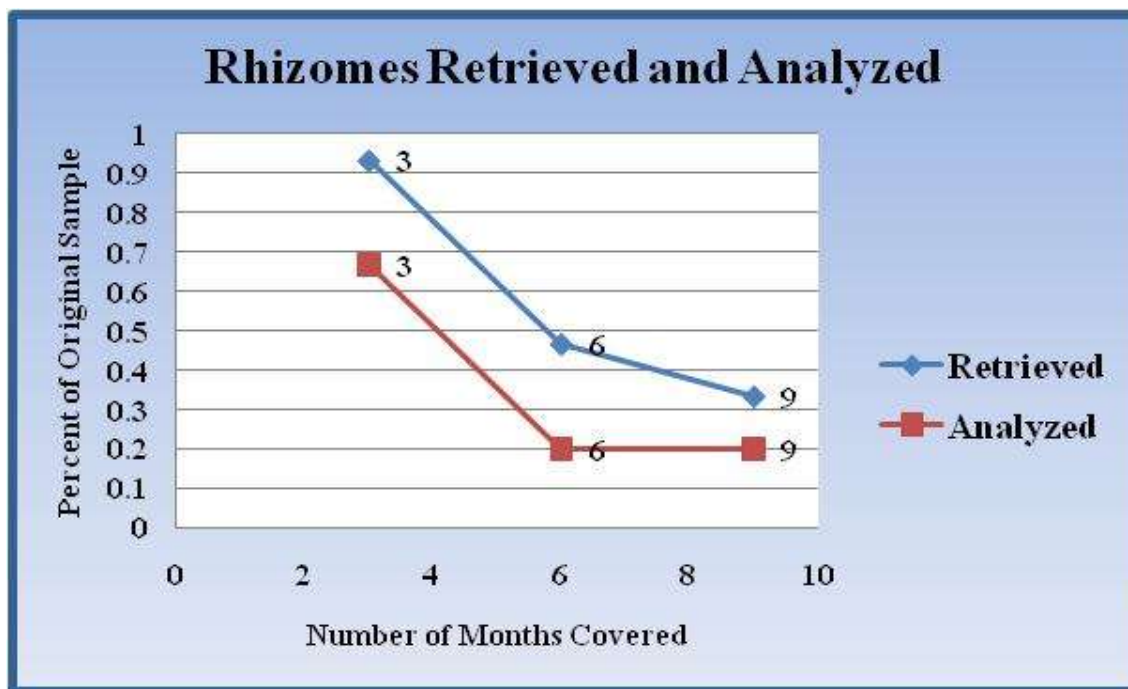


Figure 10-29. Percent Rhizomes retrieved and analyzed versus original sample.

10.8.4 Overall recommendations

Every restoration/re-vegetation project is different based on the objectives, the condition of the site and the surrounding landscape along with potential limitations for each site. A perfect example is the APP regulations one has to work with on these agricultural watercourses. In addition, treatment costs (as shown in Table 10-6) must also be considered in the solution. Therefore, the recommendations presented below should be incorporated into King County's mitigation plans with these factors in mind:

- 1) If tilling is possible on the site, till the site and cover with a thick and dense opaque material for at least one year without disturbing. This fabric should not break down for several years and should be staked down securely with stakes or mulch/hogfuel. Tilling the site will also allow for increased microtopography which will also allow for increased

species diversity (Maurer et al. 2003). After this point, one would be able to plant directly within the weed material without the cost of adding topsoil/compost.

- 2) Using hogfuel is recommended. The thick, dense, potentially allelopathic material suppressed RCG re-growth in this study.
- 3) Plant a dense multi-canopy of native vegetation. Species should be chosen which will supply multiple canopies providing dense shade for additional invasive plant control, a preferred assembly of invertebrates and habitat diversity.
- 4) Plant species that have characteristics which allow the species to compete effectively with RCG. These characteristics include: fast growing, leafing out early in the season, high leaf area index for shading.

Table 10-6. Cost of the recommended treatments for one mile of watercourse

HF/Willow		HF/RCG Barrier		Tilling/Weed Fabric/RCG Barrier	
Item	Cost	Item	Cost	Item	Cost
Mowing labor & mower	\$480	Mowing labor & mower	\$480	Mowing & tilling labor & equipment	\$960
Burlap	\$5,488	Burlap	\$5,488	Weed fabric (geotextile/ coconut & straw)	\$3,062-\$5,465
Hogfuel	\$8,790 (6'')	Hogfuel	\$8,790 (6'')	Labor for planting	\$4,930
<i>Salix</i> sp. stakes	\$4,677 (3')	Compost	\$24,000	<i>Salix</i> sp. Stakes	\$1,267 (3')
Labor for planting	\$700	Labor for planting	\$4,930	<i>S. microcarpus</i> plugs	\$1,350
		<i>Salix</i> sp. stakes	\$1,267 (3')	<i>R. spectabilis</i> 2 gallon (36 inch)	\$880
		<i>S. microcarpus</i> plugs	\$1,350	<i>L. involucrata</i> 2 gallon (36 inch)	\$880
		<i>R. spectabilis</i> 2 gallon (36 inch)	\$880	<i>S. albus</i> – 1 gallon (18 inch)	\$968
		<i>L. involucrata</i> 2 gallon (36 inch)	\$880	<i>R. parviflorus</i> – 1 gallon (18 inch)	\$880
		<i>S. albus</i> – 1 gallon (18 inch)	\$968	<i>C. sericea</i> – 3 foot stakes	\$704
		<i>R. parviflorus</i> – 1 gallon (18 inch)	\$880	<i>R. bracteosum</i> – 2 gallon (36 inch)	\$1,056
		<i>C. sericea</i> – 3 foot stakes	\$704		
		<i>R. bracteosum</i> – 2 gallon (36 inch)	\$1,056		

10.9 References

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Appendix 10-A –

Principal Project Layout, Quigley (Horticulture site)

½ meter between plots and one meter between replicates

Each plot is 2 meters wide

Replicate 1 (western most plot)

Control	RCG Barrier	Red Cedar Hogfuel / Willows	Red Cedar Hogfuel / RCG Barrier
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3 meters in length

Replicate 2

Control	Red Cedar Hogfuel / RCG Barrier	RCG Barrier	Red Cedar Hogfuel / Willows
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Replicate 3

RCG Barrier	Red Cedar Hogfuel / Willows	Red Cedar Hogfuel / RCG Barrier	Control
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Pilot Project Layout

Each plot is 1.5 meters by 1.5 meters

Replicate 1

Steam Salal	No Steam Shade	Steam Mulch	Steam Control	No Steam Clover	No Steam Control	No Steam Salal	No Steam Mulch	Steam Shade	Steam Clover
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Replicate 2

No Steam Clover	Steam Control	No Steam Mulch	Steam Mulch	No Steam Salal	No Steam Shade	Steam Clover	Steam Salal	No Steam Control	Steam Shade
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Replicate 3

No Steam Salal	Steam Shade	No Steam Mulch	No Steam Shade	Steam Salal	Steam Control	Steam Mulch	No Steam Clover	Steam Clover	No Steam Control
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(All plots are aligned linearly along the watercourse noted in the perspective maps on pages 10-12 to 10-13)

Appendix 10-B –

Principal Project Layout, Calhoon (Livestock site)

½ meter between plots and one
meter between replicates

← Each plot is 2
meters wide →

Replicate 1 (eastern most plot)

Control	Red Cedar Hogfuel / RCG Barrier	RCG Barrier	Red Cedar Hogfuel / Willows
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1 meter
in length
↓

Replicate 2

Red Cedar Hogfuel / RCG Barrier	Red Cedar Hogfuel / Willows	Control	RCG Barrier
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Replicate 3

Red Cedar Hogfuel / RCG Barrier	RCG Barrier	Red Cedar Hogfuel / Willows	Control
---------------------------------	-------------	-----------------------------	---------

(All plots are aligned linearly along the watercourse noted in the perspective maps on pages 10-12 to 10-13)

Appendix 10-C–

Principal Project Layout, Olney (Natural site)

½ meter between plots and one
meter between replicates

← Each plot is 2
meters wide →

Replicate 1 (southern most plot)

RCG Barrier	Red Cedar Hogfuel / RCG Barrier	Red Cedar Hogfuel / Willows	Control
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3 meters
in length

Replicate 2

Red Cedar Hogfuel / Willows	Control	Red Cedar Hogfuel / RCG Barrier	RCG Barrier
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Replicate 3

Control	Red Cedar Hogfuel / Willows	RCG Barrier	Red Cedar Hogfuel / RCG Barrier
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(All plots are aligned linearly along the watercourse noted in the perspective maps on pages 10-12 to 10-13)